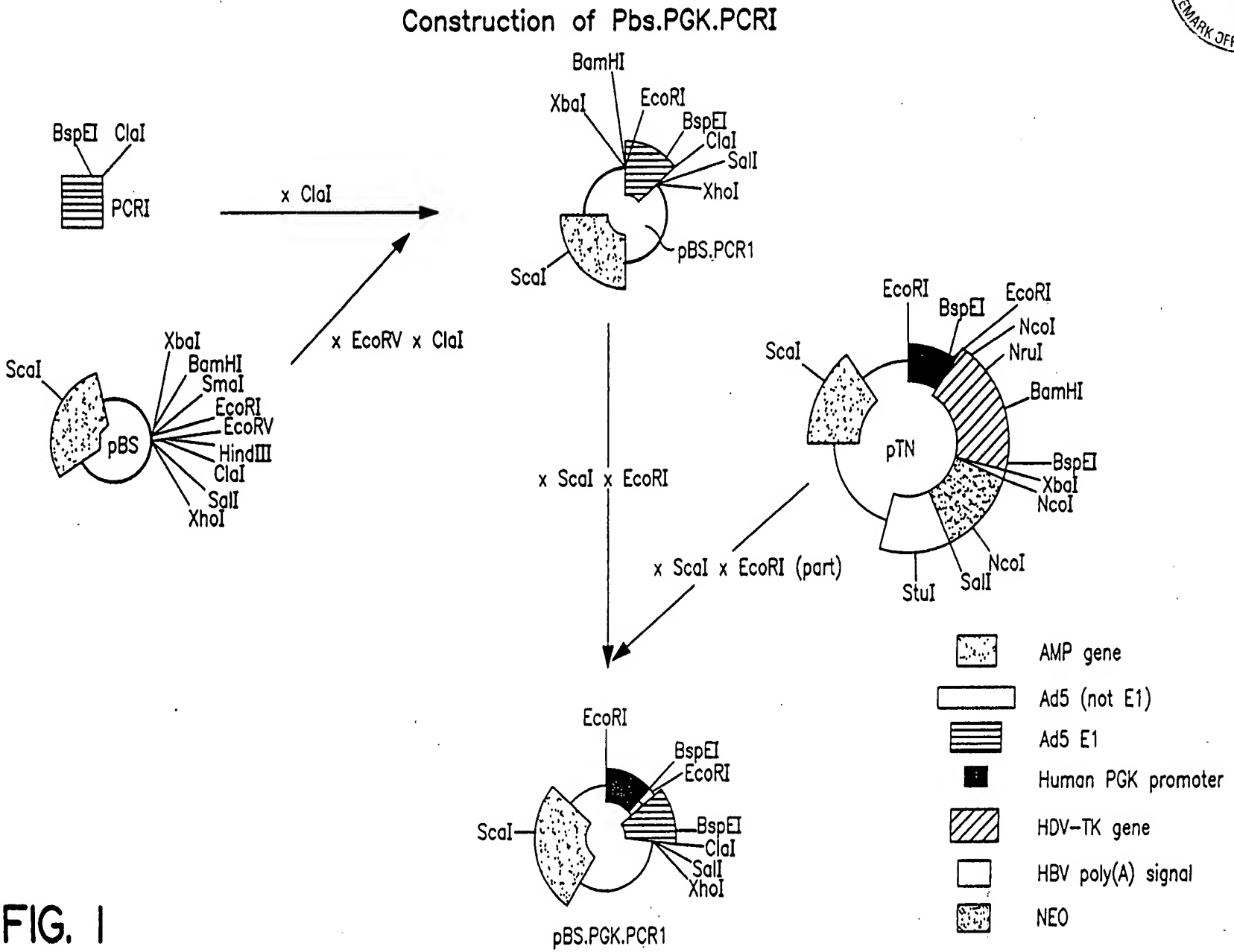


FIG. 1

Construction of pIG.E1a.E1b.X

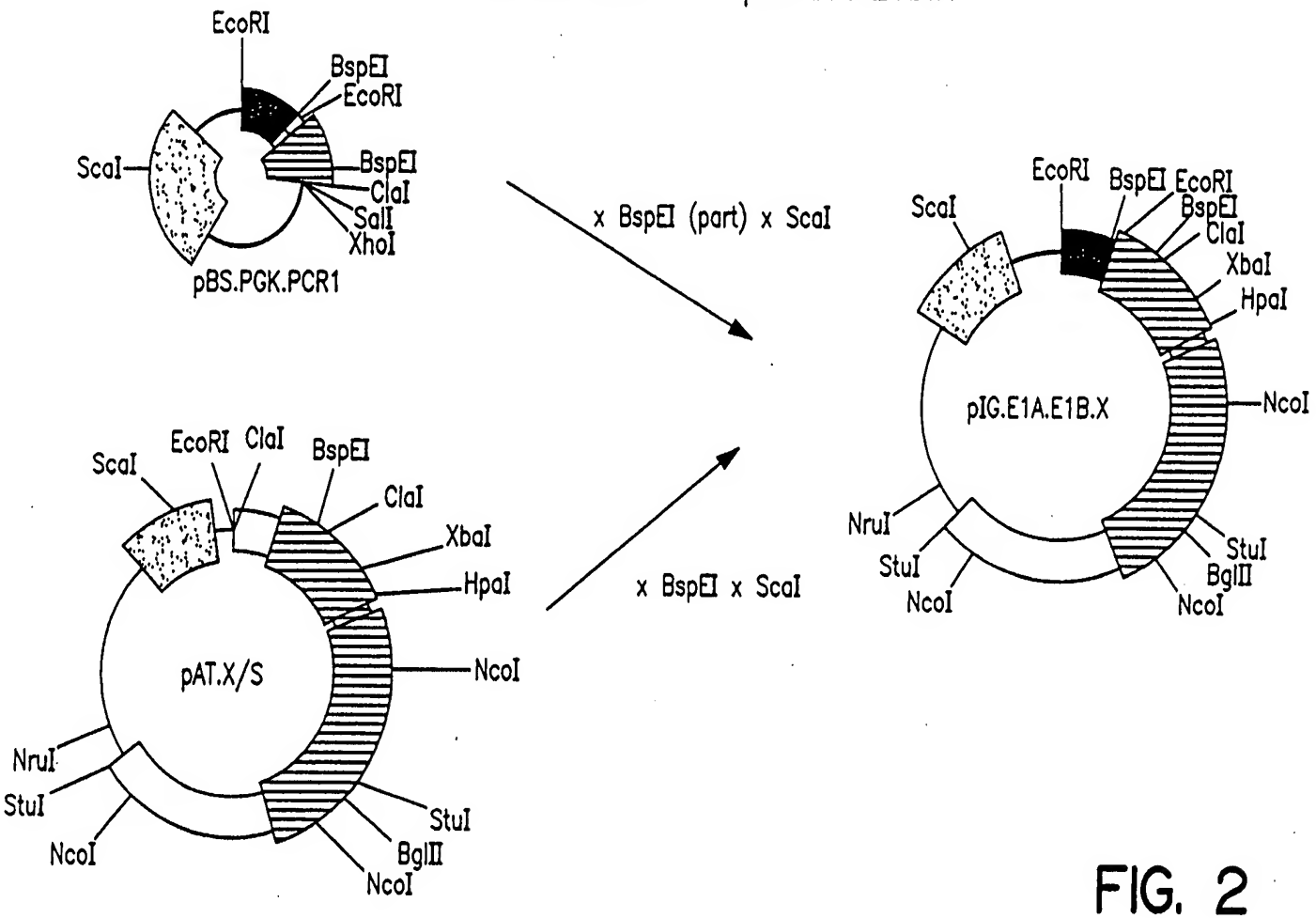


FIG. 2

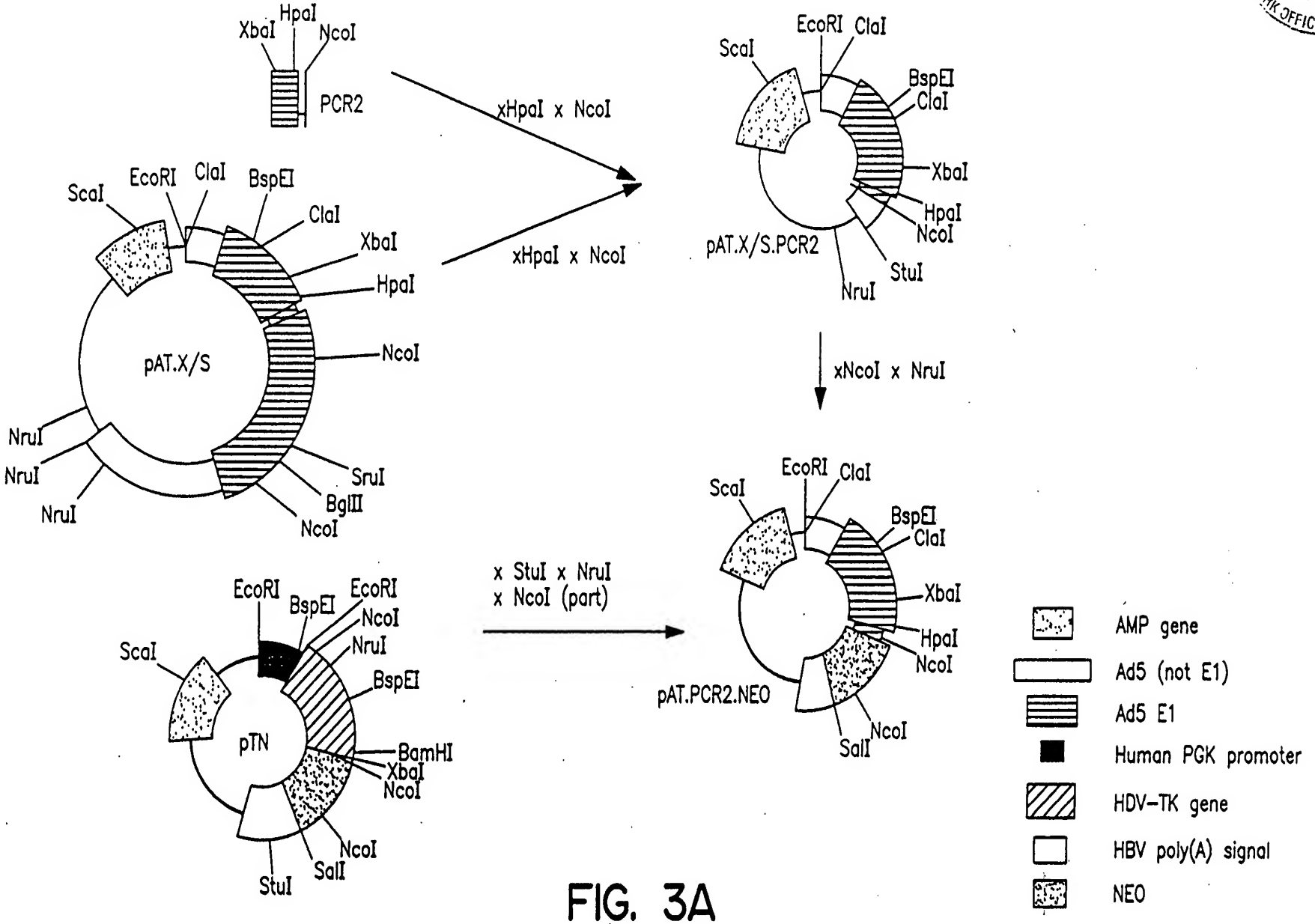


FIG. 3A

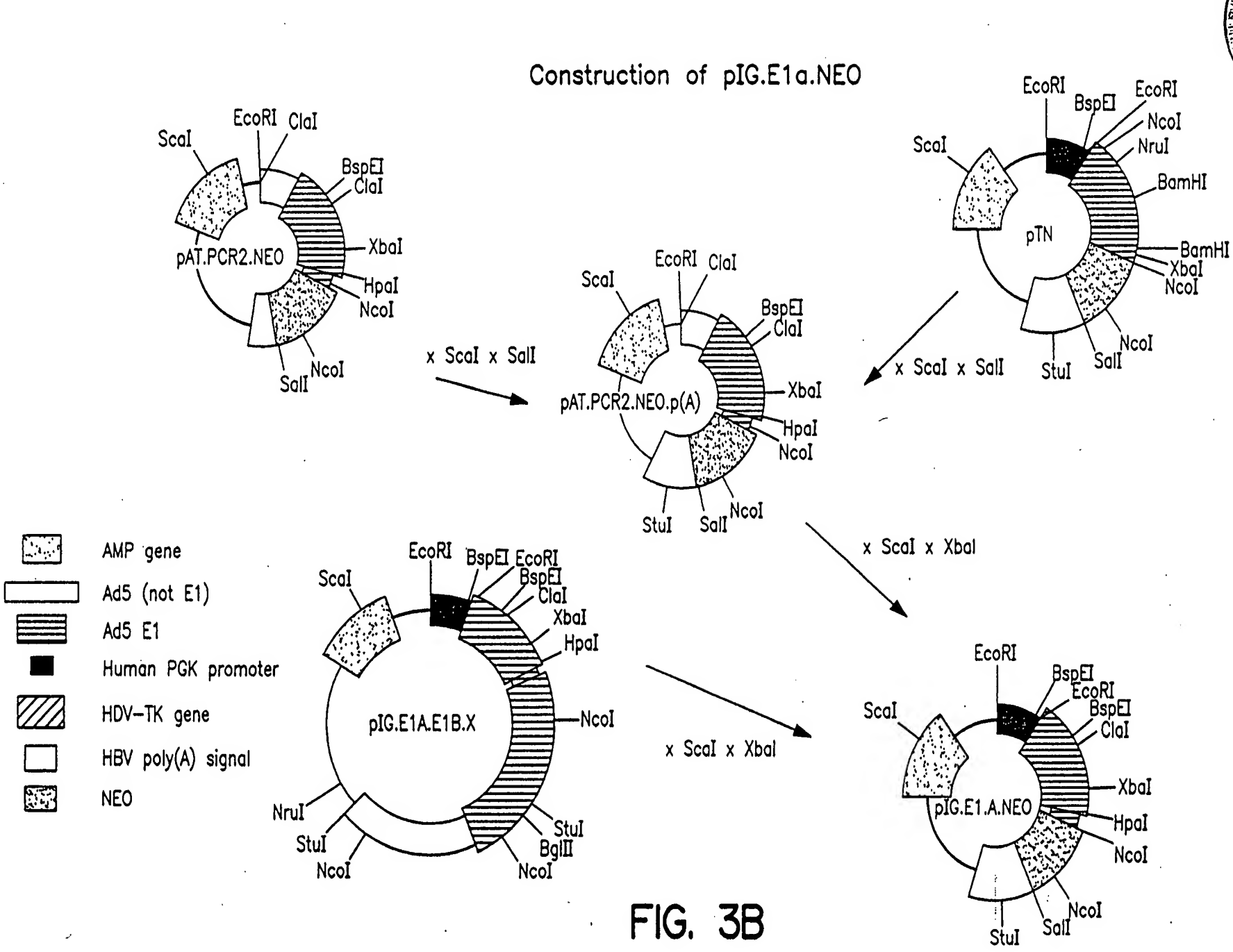
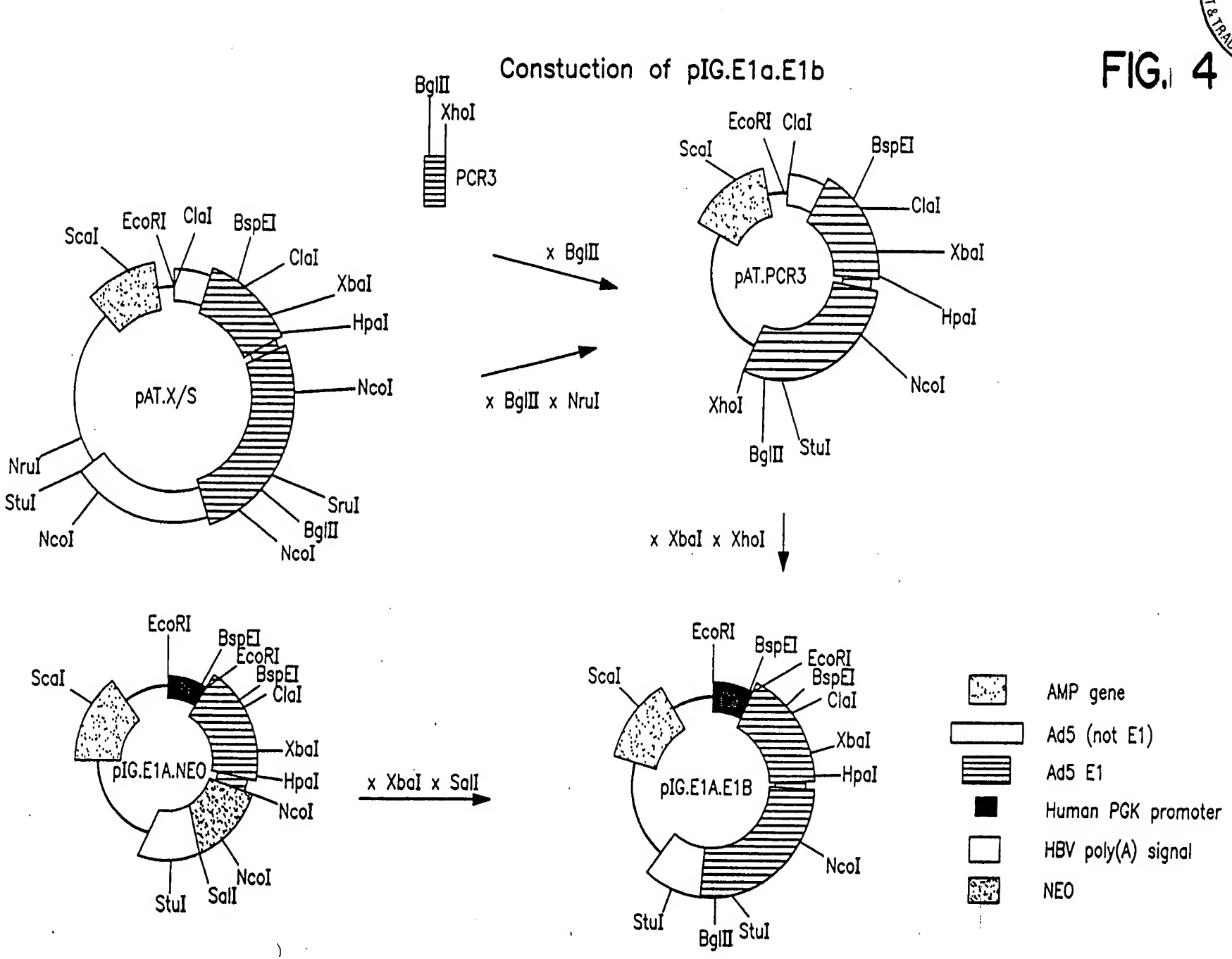


FIG. 3B

FIG. 4



Construction of pIG.NEO

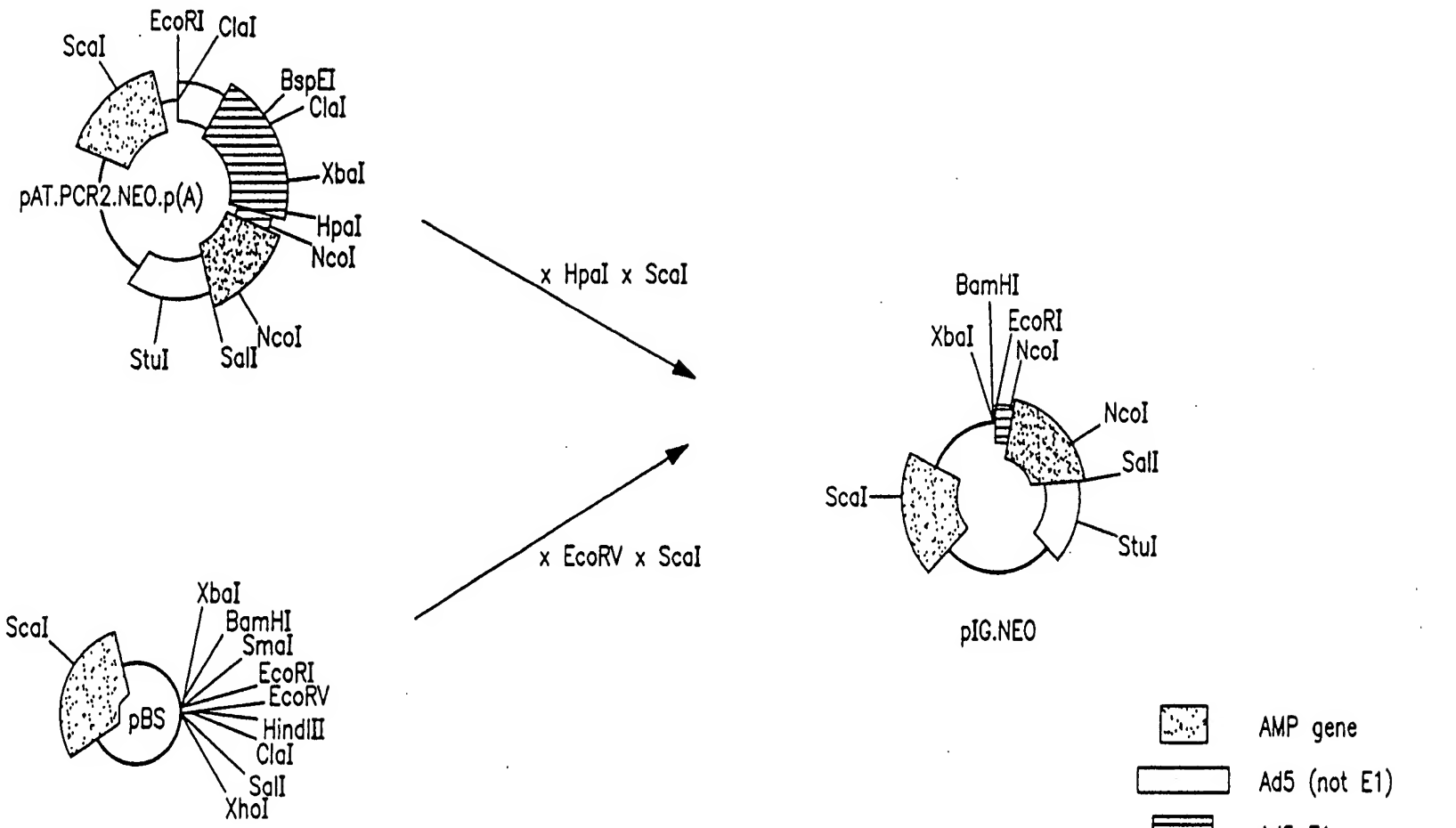
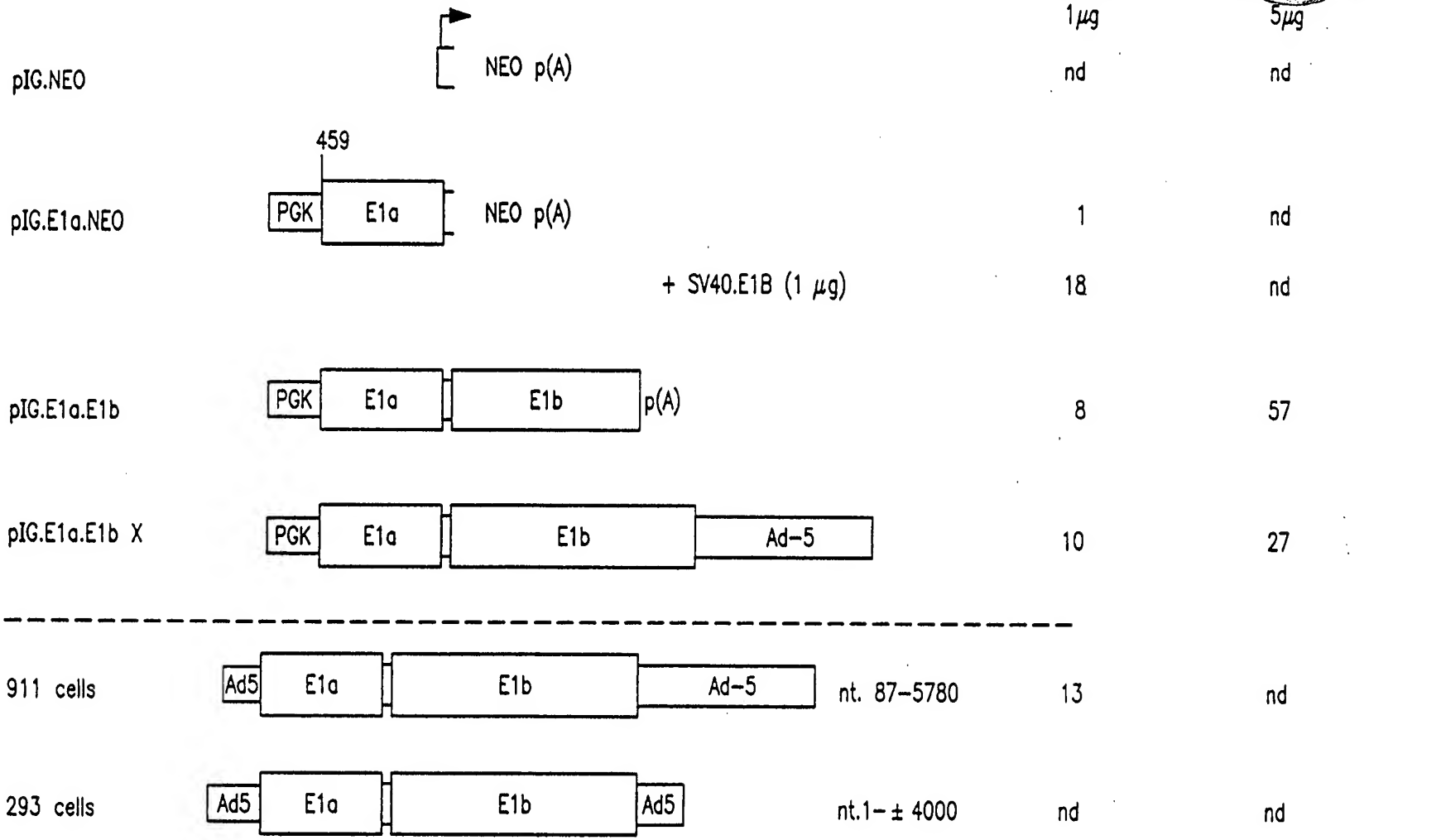


FIG. 5

Overview of available adenovirus packaging constructs and assessment of their capacity to transform primary kidney cells



*average of 5 plates 21 days after transelection

FIG. 6

Western blotting analysis of A549 clones transfected with pIG.E1A.NEO and PER clones
(HER cells transfected with pIG.E1A.E1B)

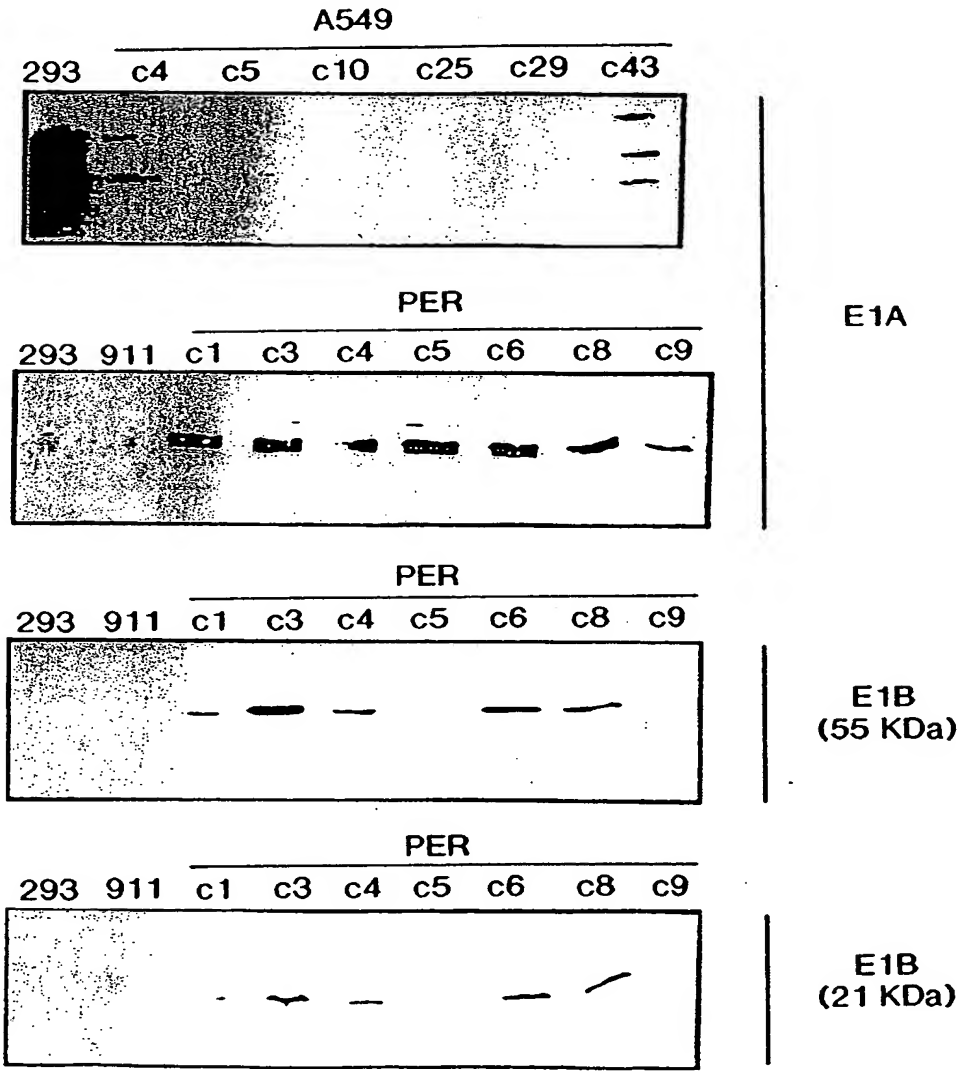


FIG. 7

Southern blot analyses of 293, 911 and PER cell lines

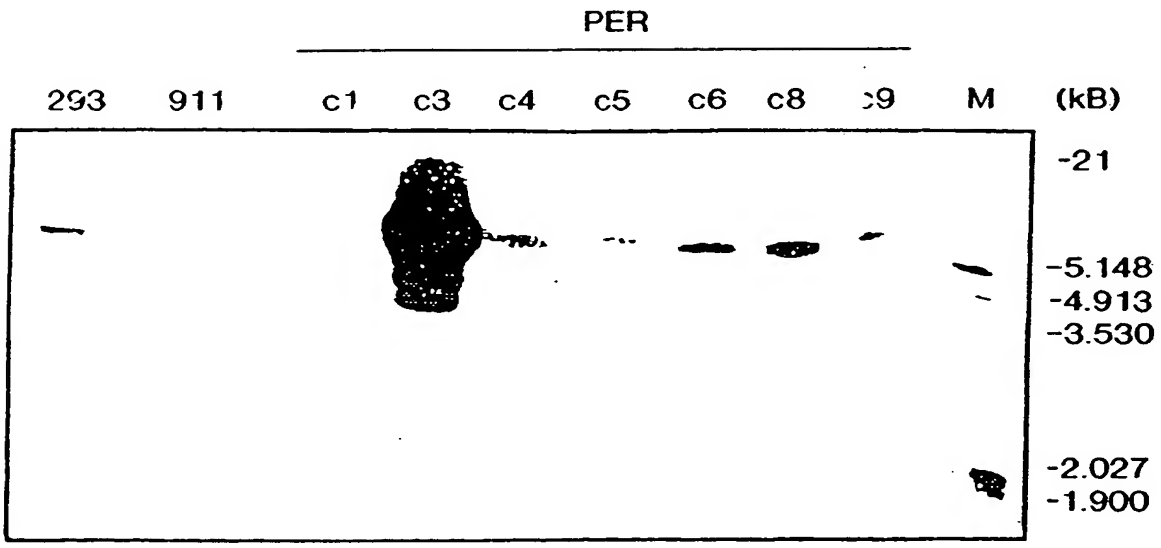


FIG. 8

Transfection efficiency of PER.C3, PER.C5, PER.C6 and 911 cells. Cells were cultured in 6-well plates and transfected (n=2) with 5 µg pRSV.lasZ by calcium-phosphate co-precipitation. Forty-eight hours later the cells were stained with X-GAL. The mean percentage of blue cells is shown.

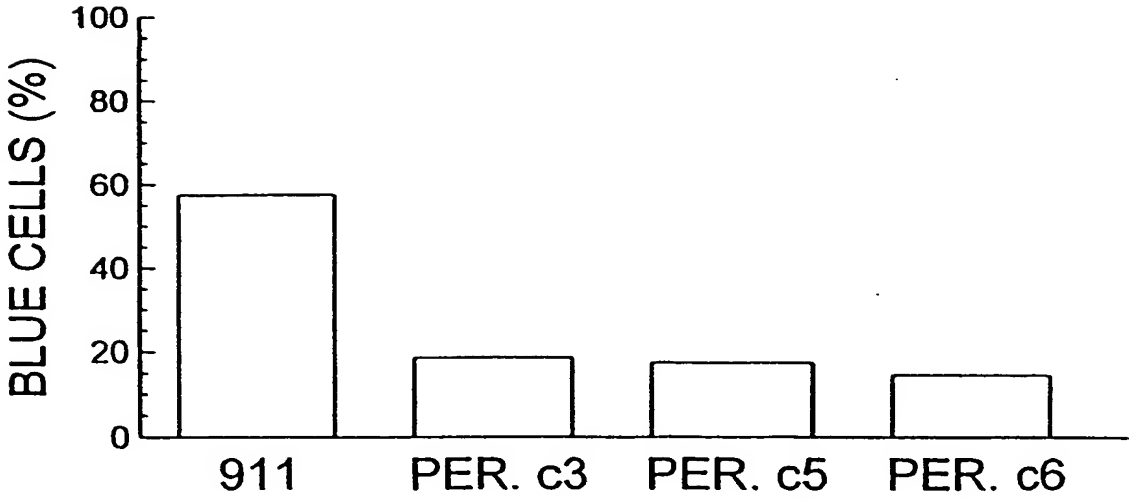


FIG. 9

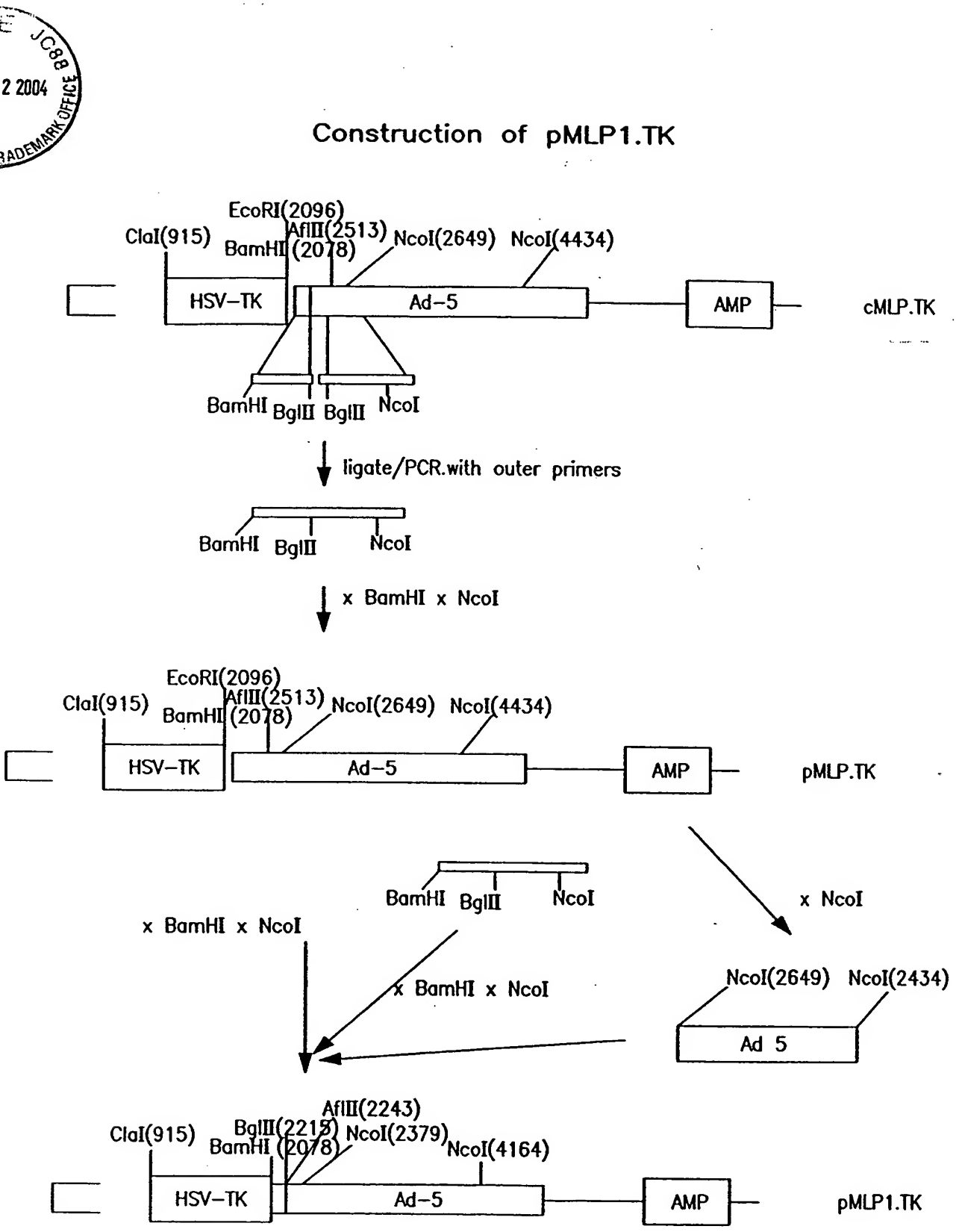


FIG. 10

New recombinant adenoviruses and packaging constructs without sequence overlap

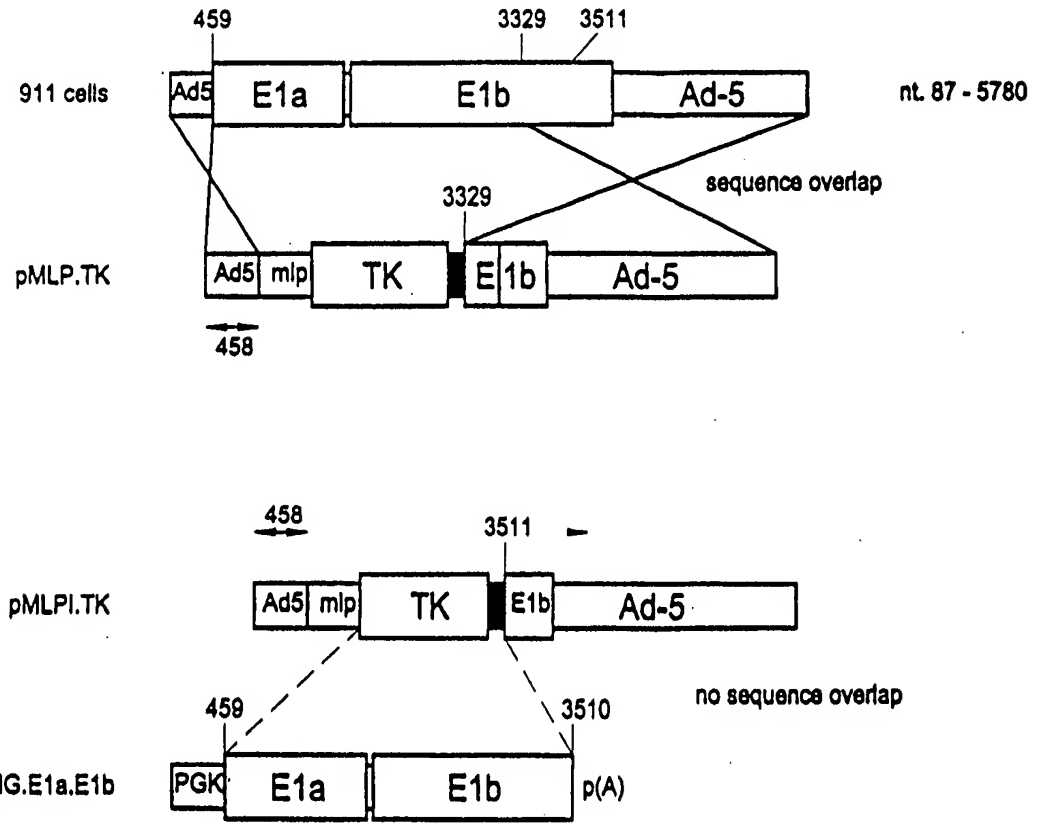
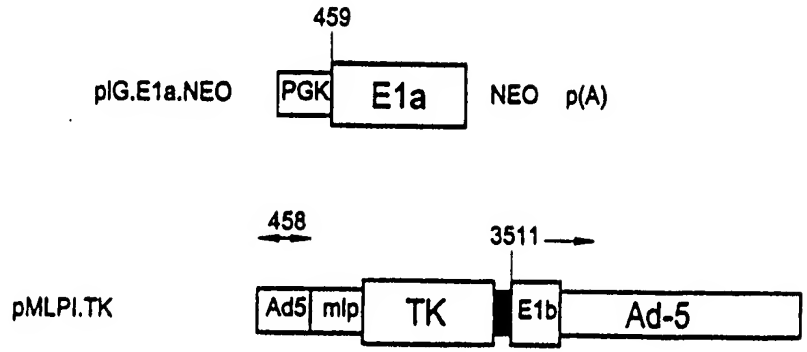


FIG. 1 IA

Packaging system based on primary cells

New recombinant adenoviruses and packaging constructs without sequence overlap



Packaging system based on established cell lines: transfection with E1a and selection with G418

FIG. 1 IB

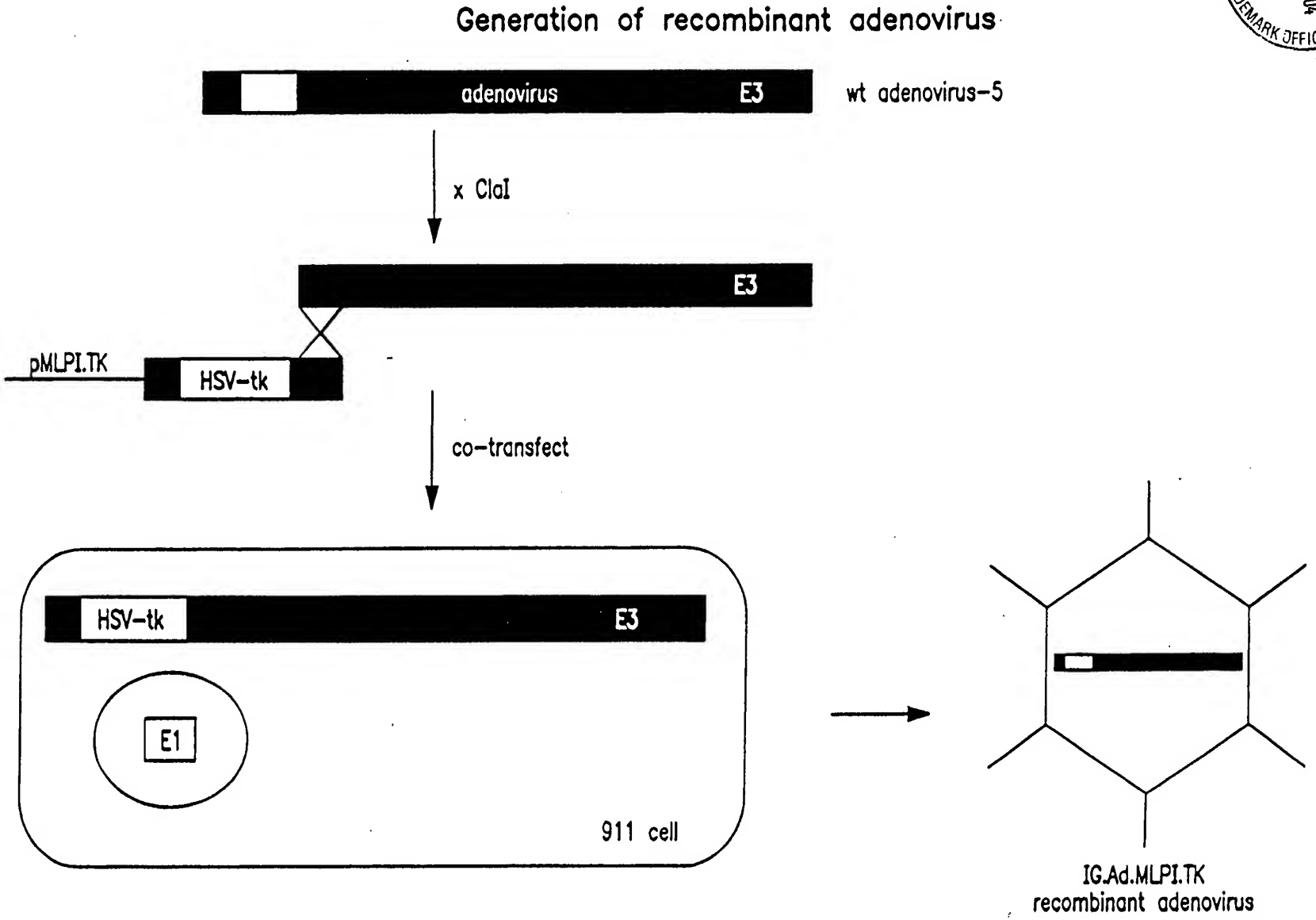


FIG. 12

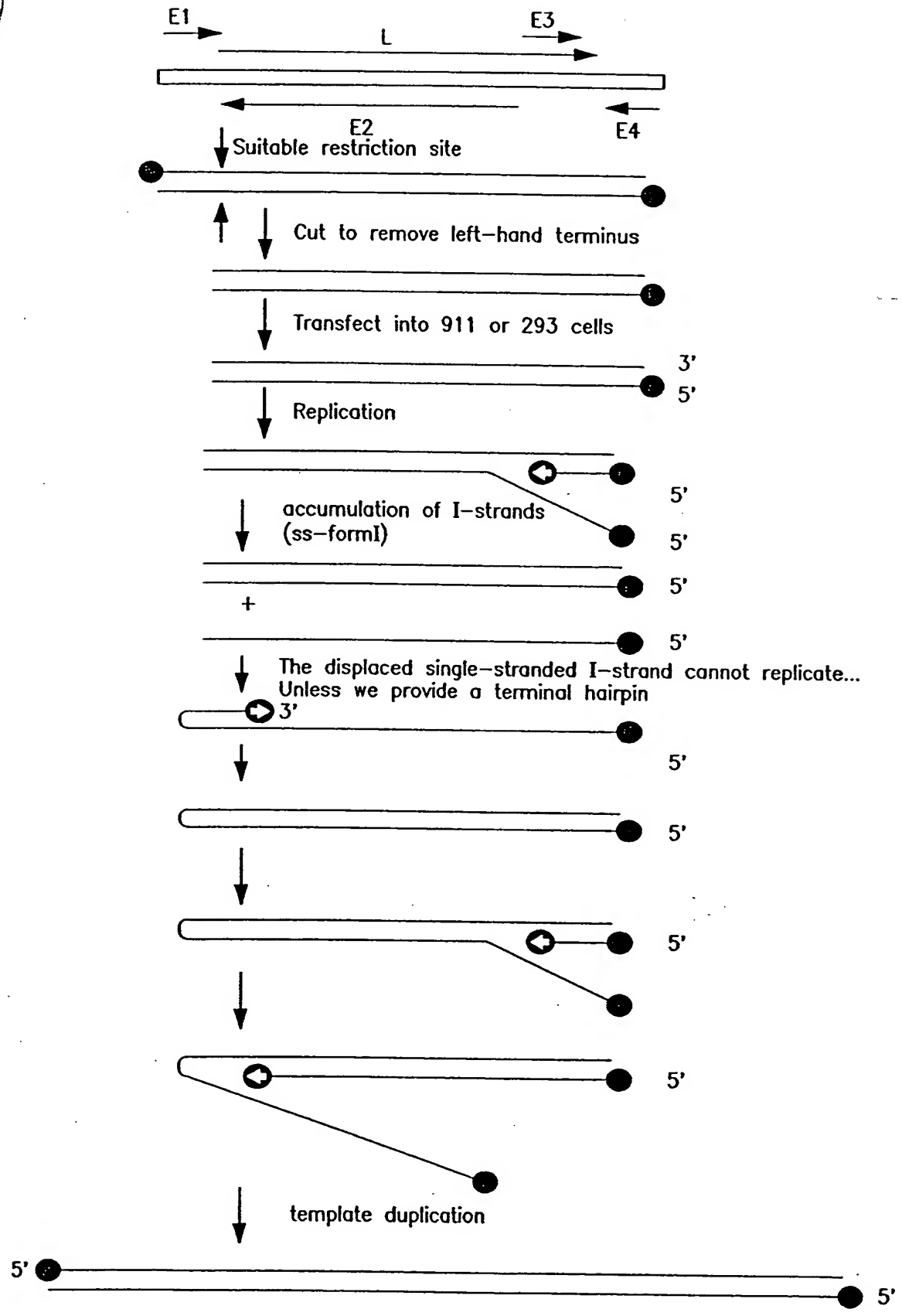


FIG. 13

Replication of Adenovirus

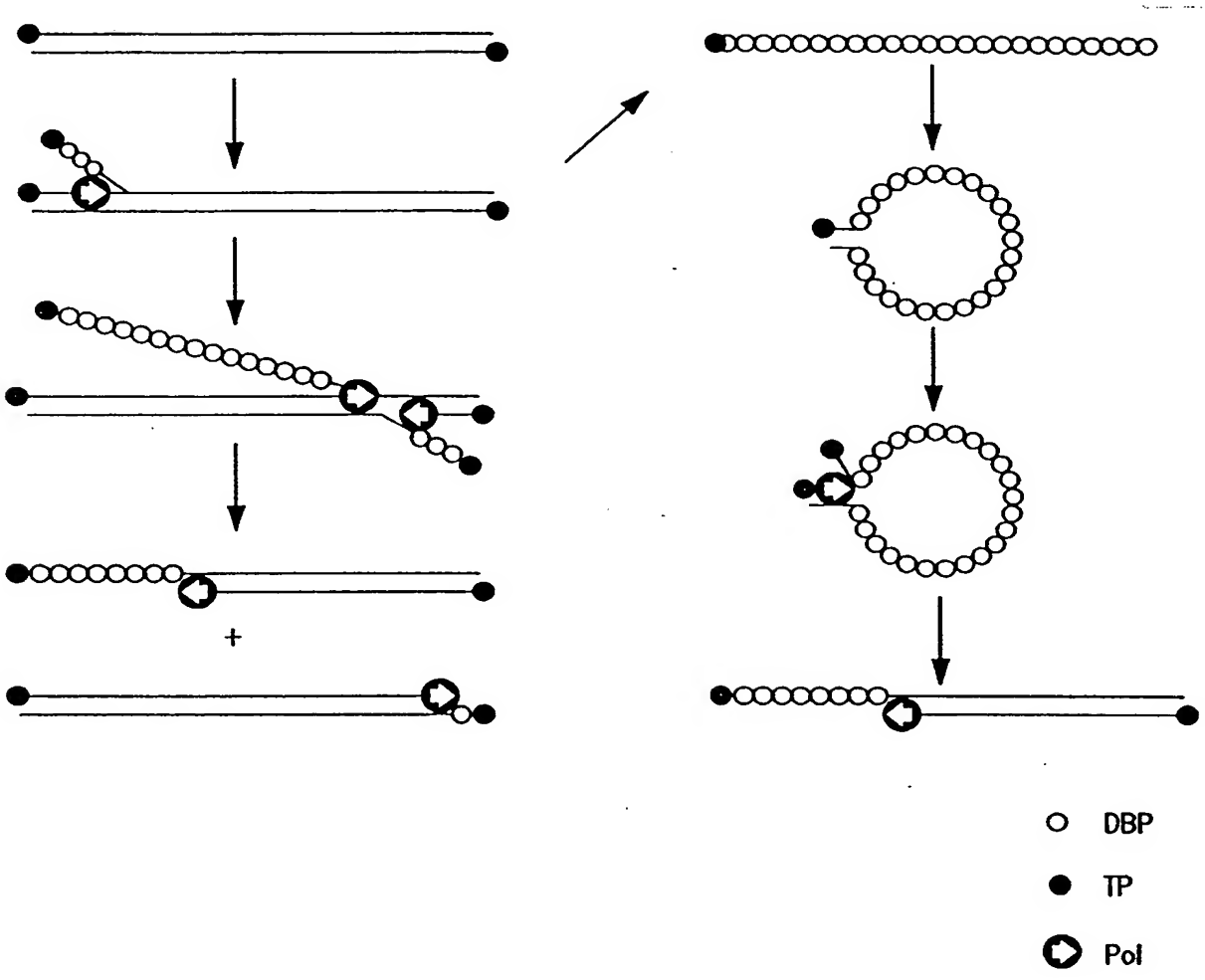


FIG. 14

The potential hairpin conformation of a single-stranded DNA molecule that contains the HP/asp sequences used in these studies. Restriction with the restriction endonucleases *Asp718I* of plasid pICLHa, containing the annealed oligonucleotide pair HP/asp1 en HP/asp2 will yield a linear double-stranded DNA fragment. In cells in which the required adenovirus genes are present, replication can initiate at the terminus that contains the ITR sequence. During the chain elongation, the one of the strands will be displaced. The terminus of the single-stranded displaced-strand molecule can adopt the conformation depicted above. In this conformation the free 3'-terminus can serve as a primer for the cellular and/or adenovirus DNA polymerase, resulting in conversion of the displaced strand in a double-stranded form.

```
5'-GTACACTGACCTAGTGCCGCCCGGGCA
                  |||||||||||||||||| A
            3'-GATCACGGCGGGCCCGA
```

FIG. 15

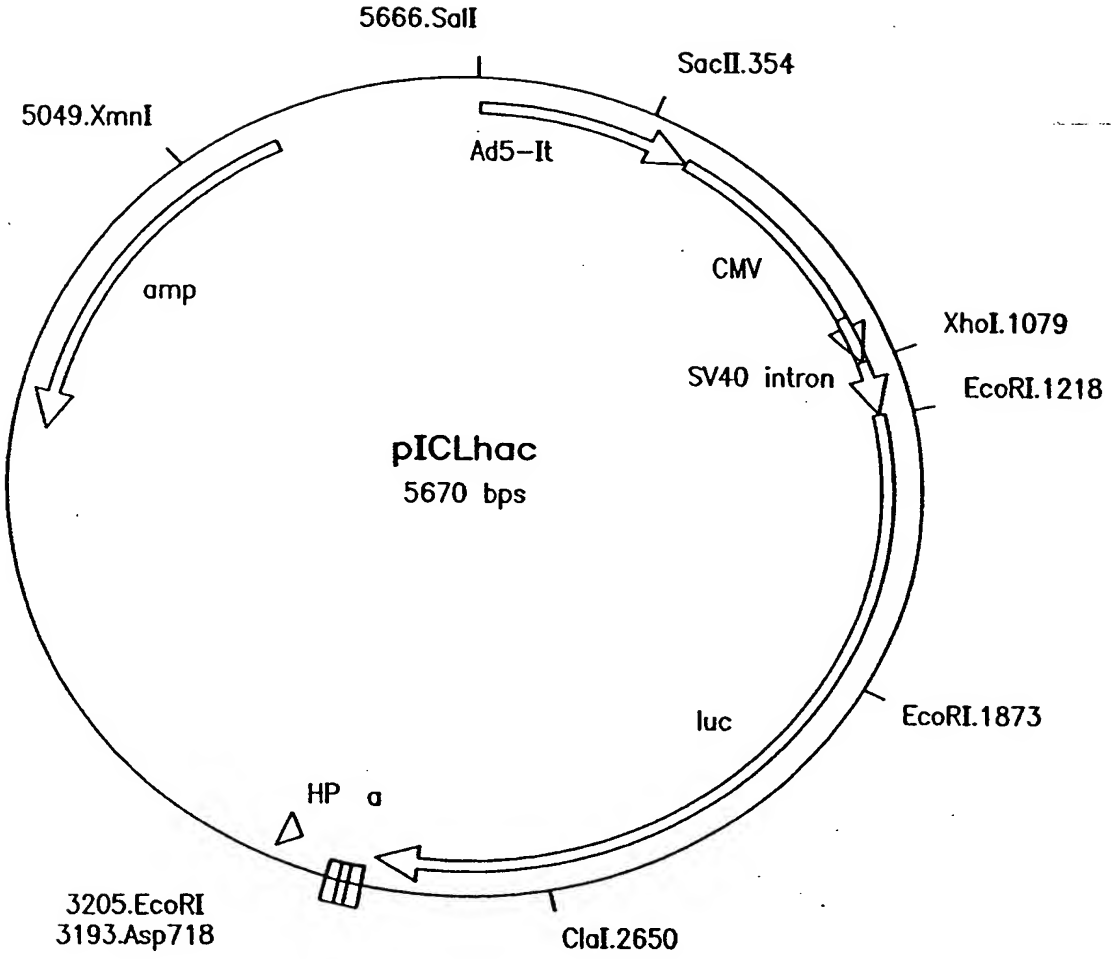


FIG. 16

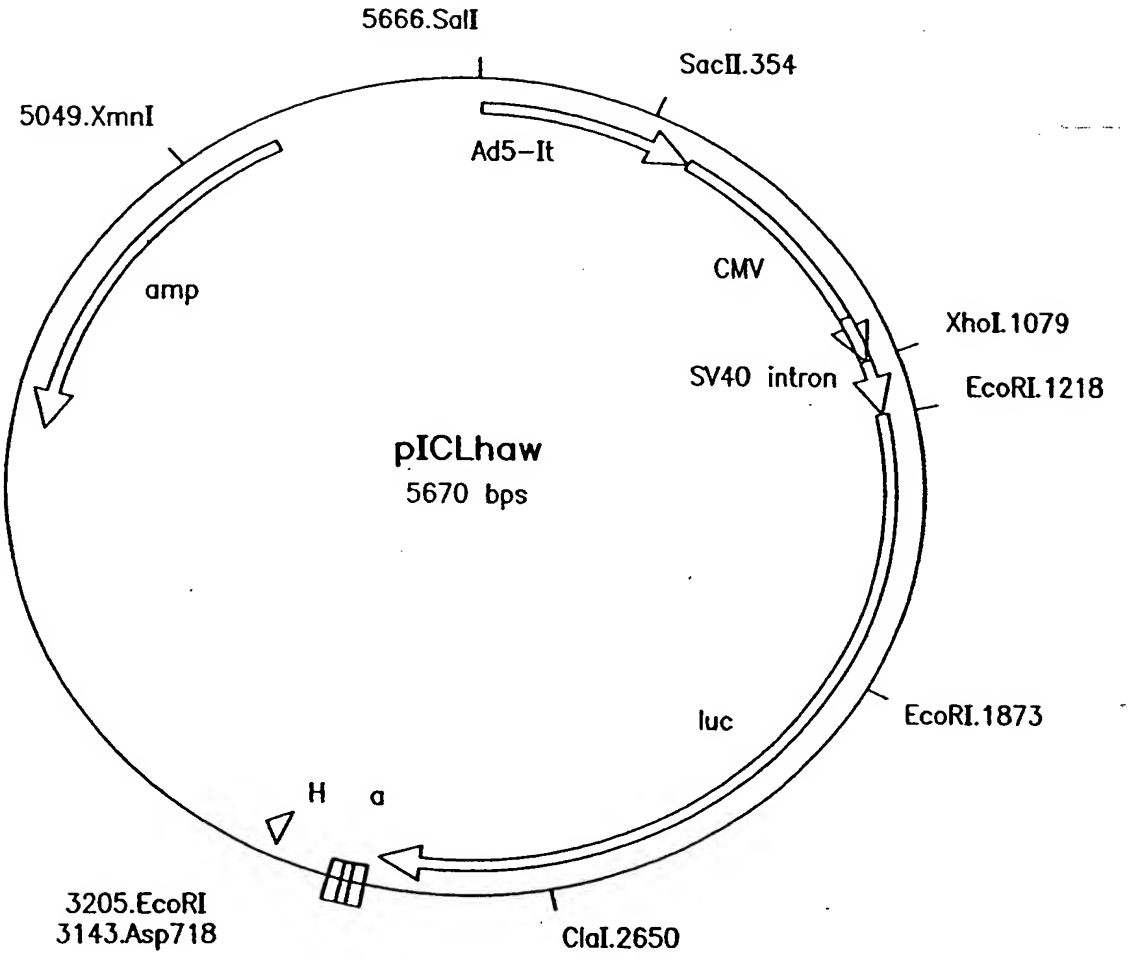


FIG. 17

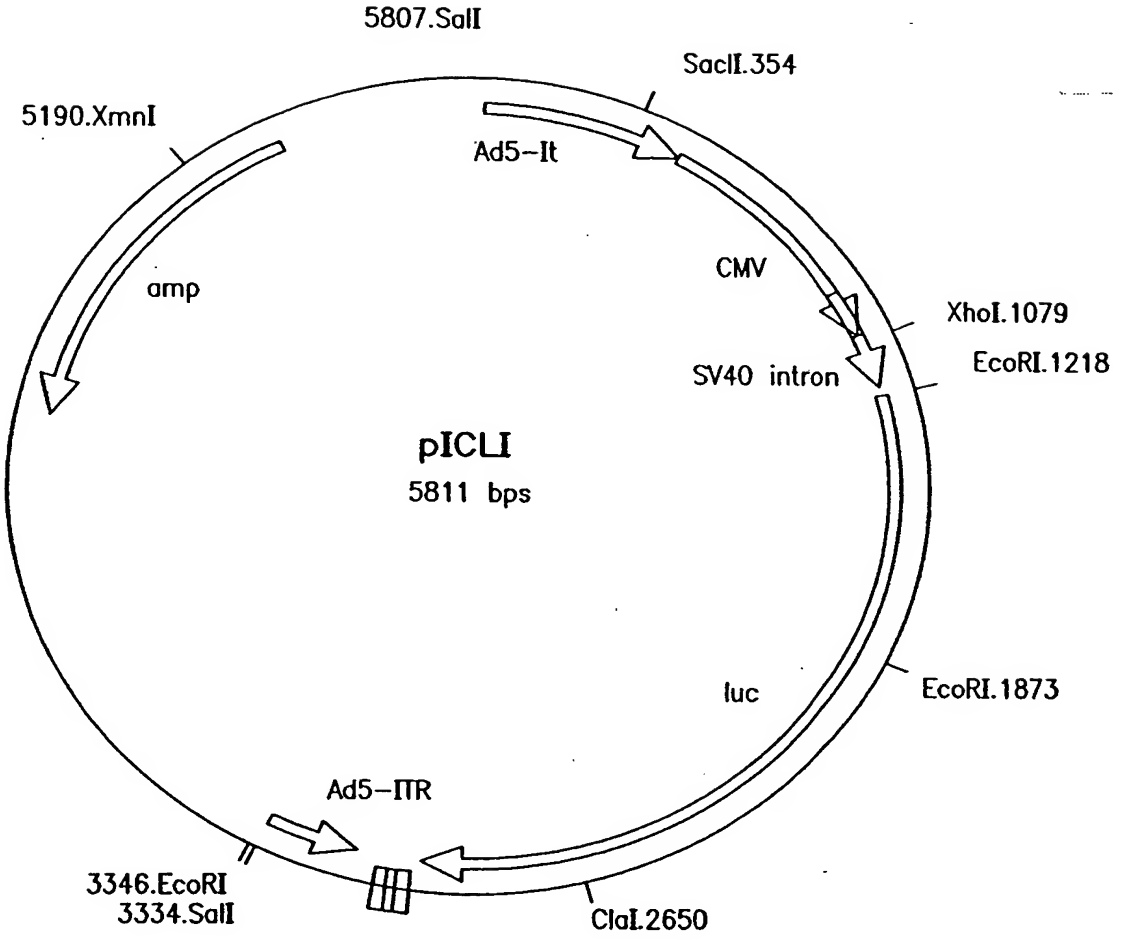


FIG. 18

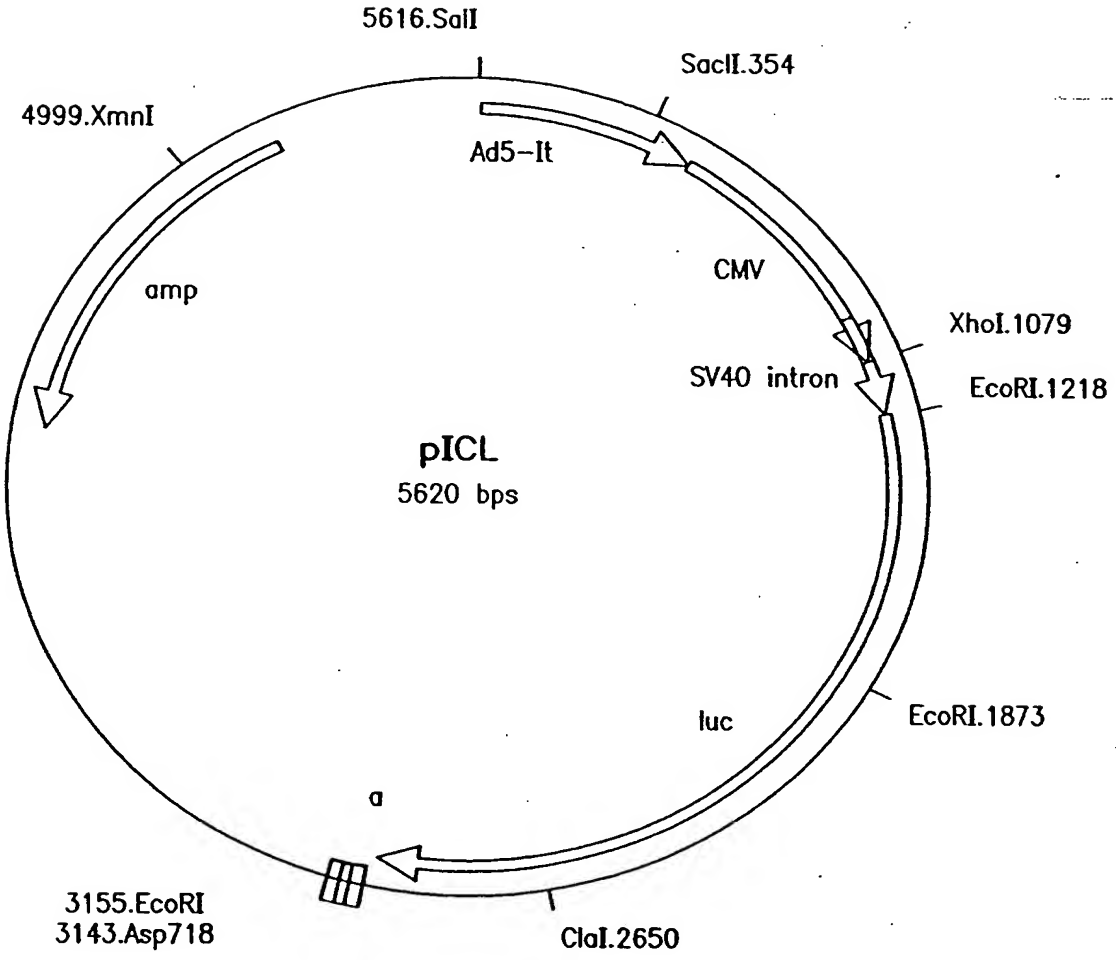


FIG. 19

Cloned adenovirous fragments

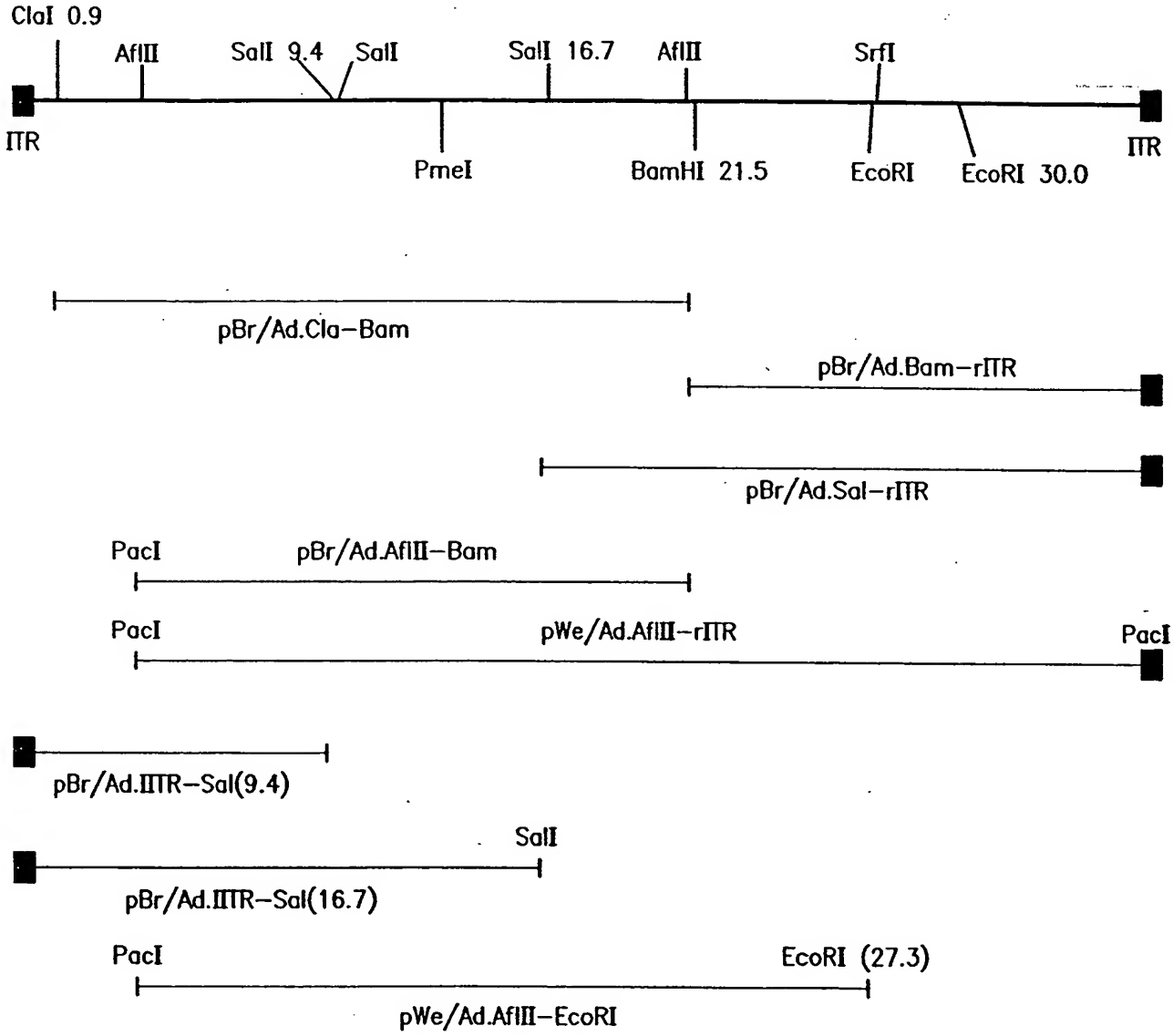


FIG. 20

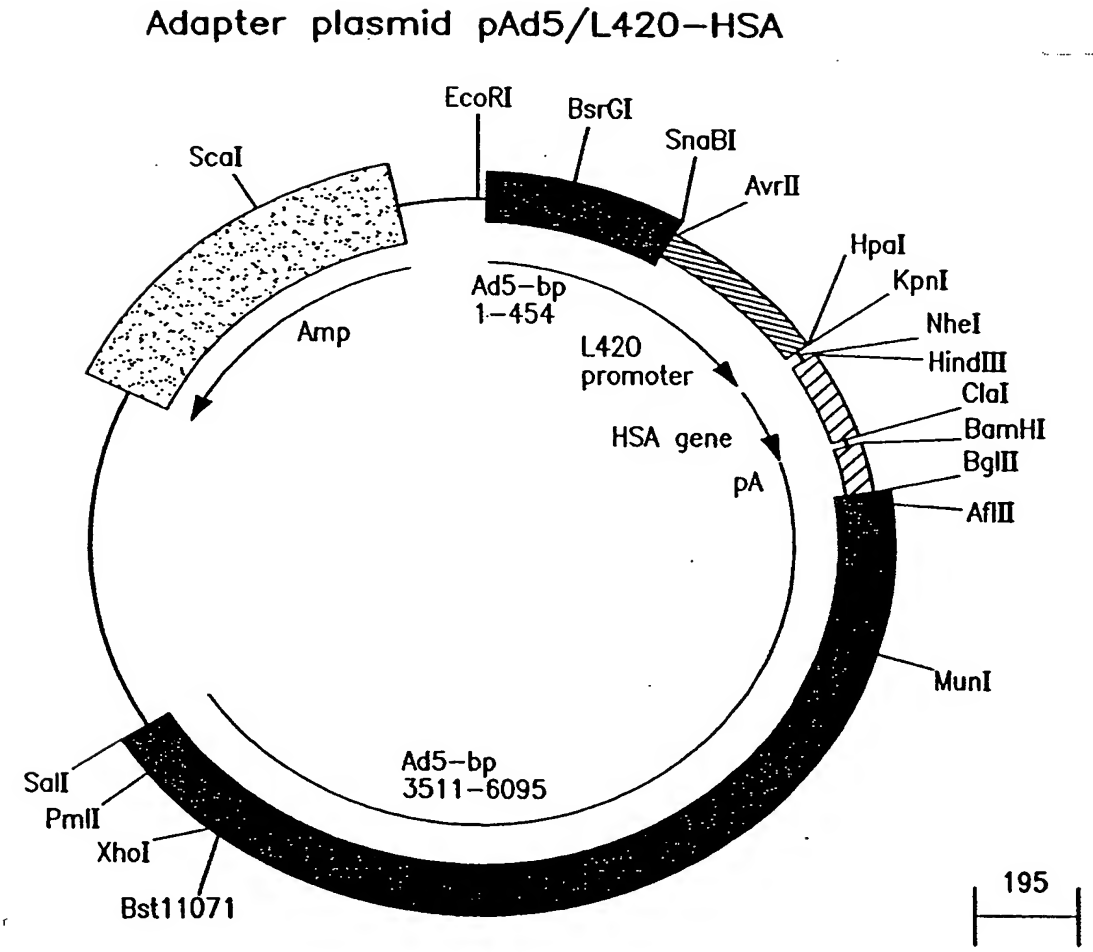


FIG. 21

Adapter plasmid pAd5/CLIP

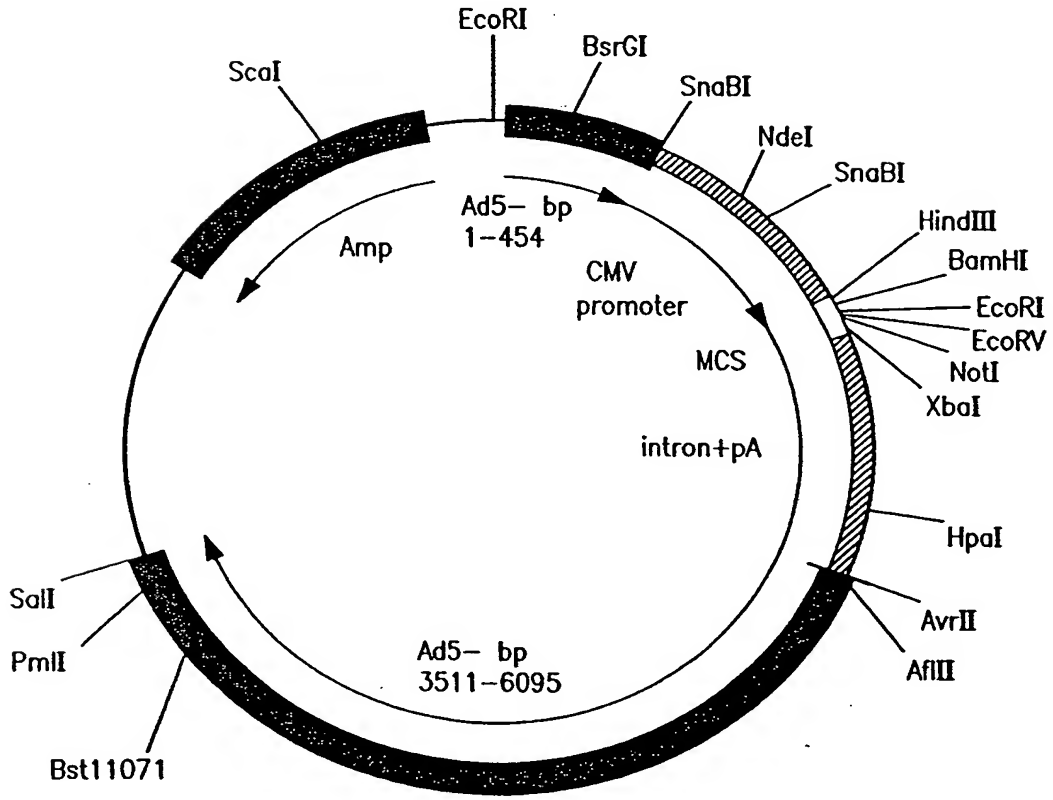


FIG. 22

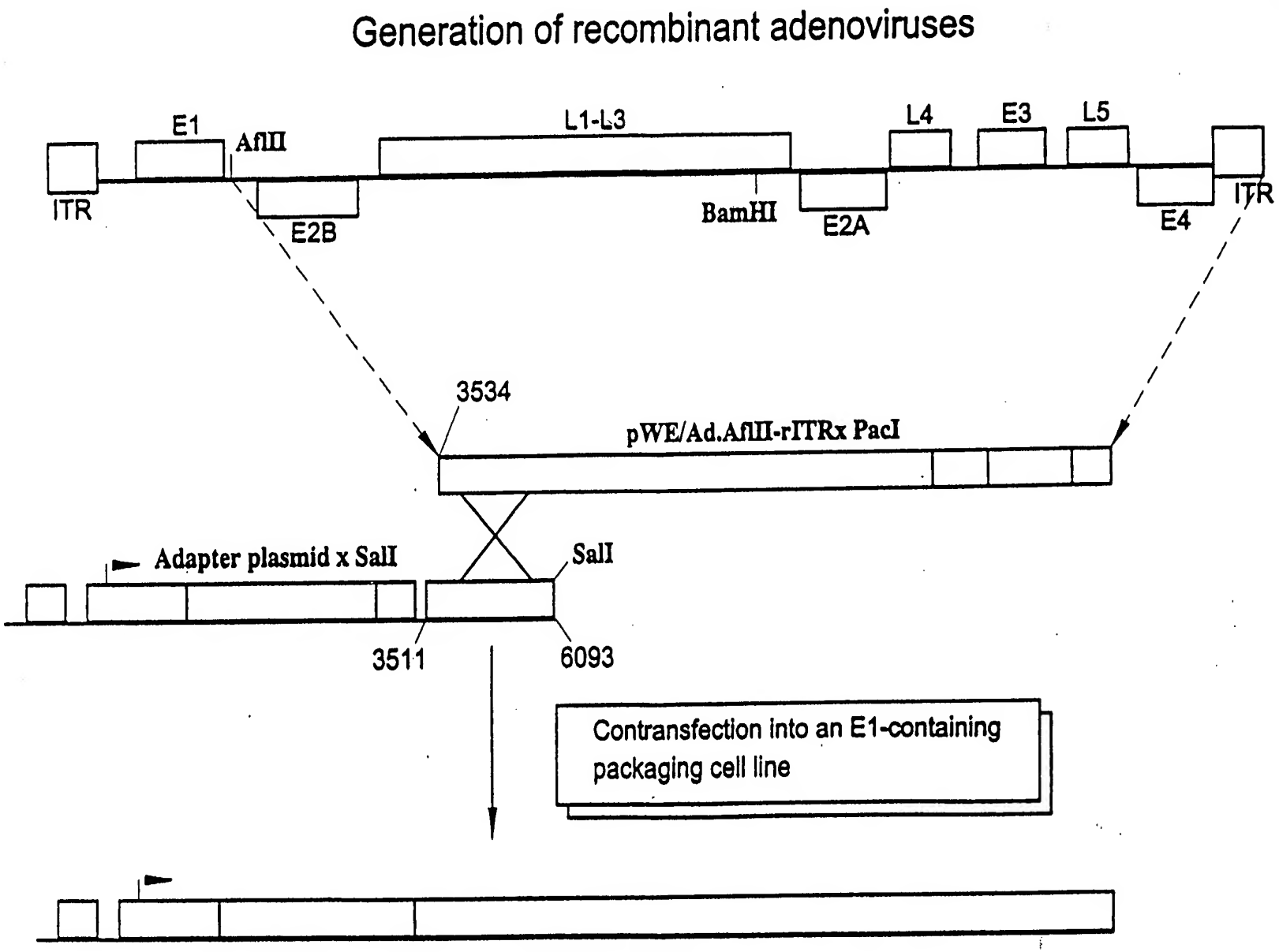


FIG. 23

Minimal adenovirus vector pMV/L420H

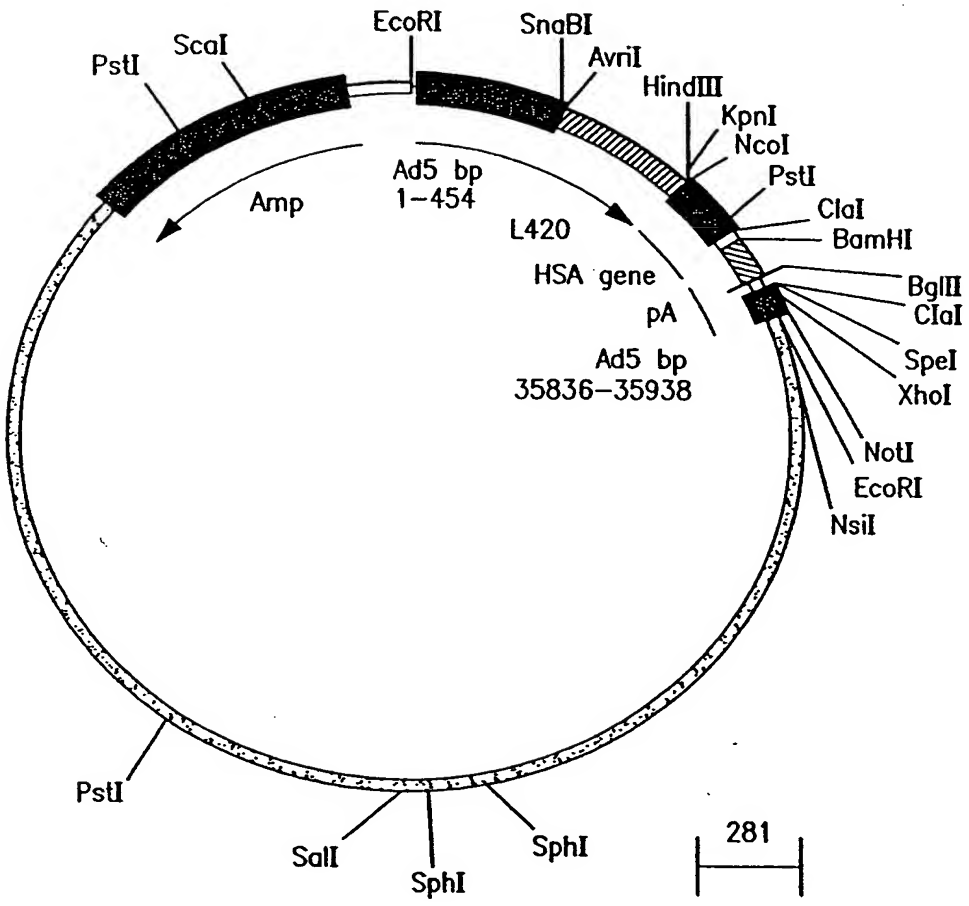


FIG. 24

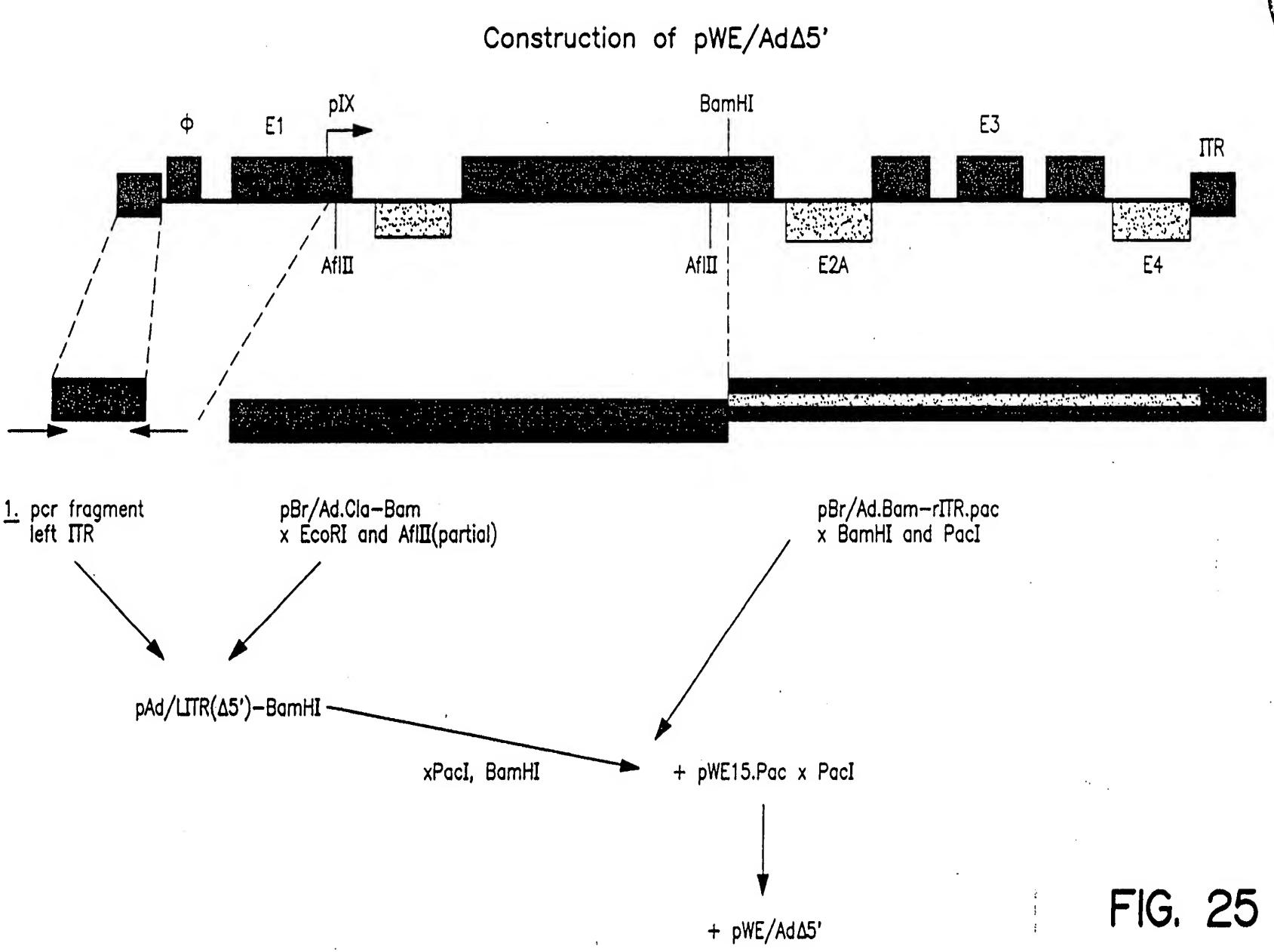


FIG. 25

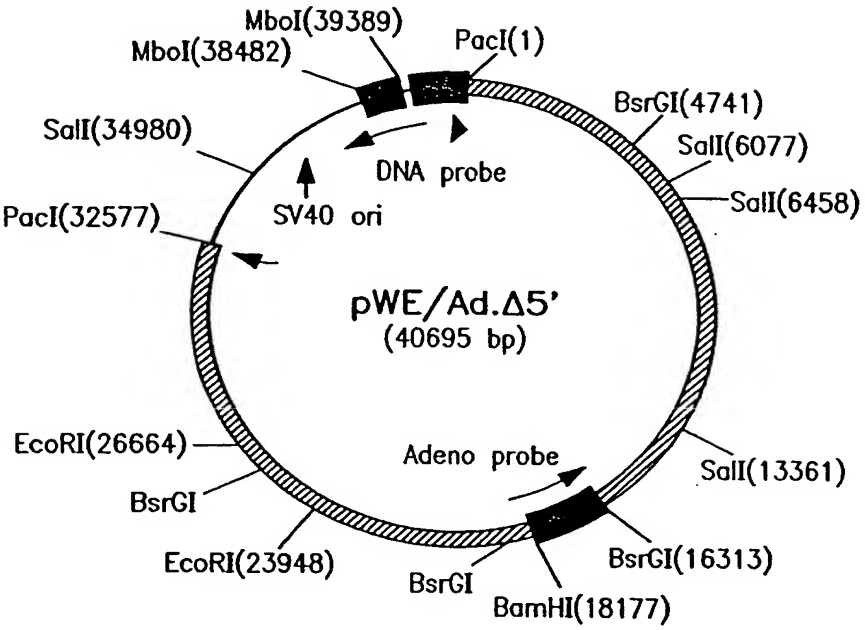


FIG. 26A

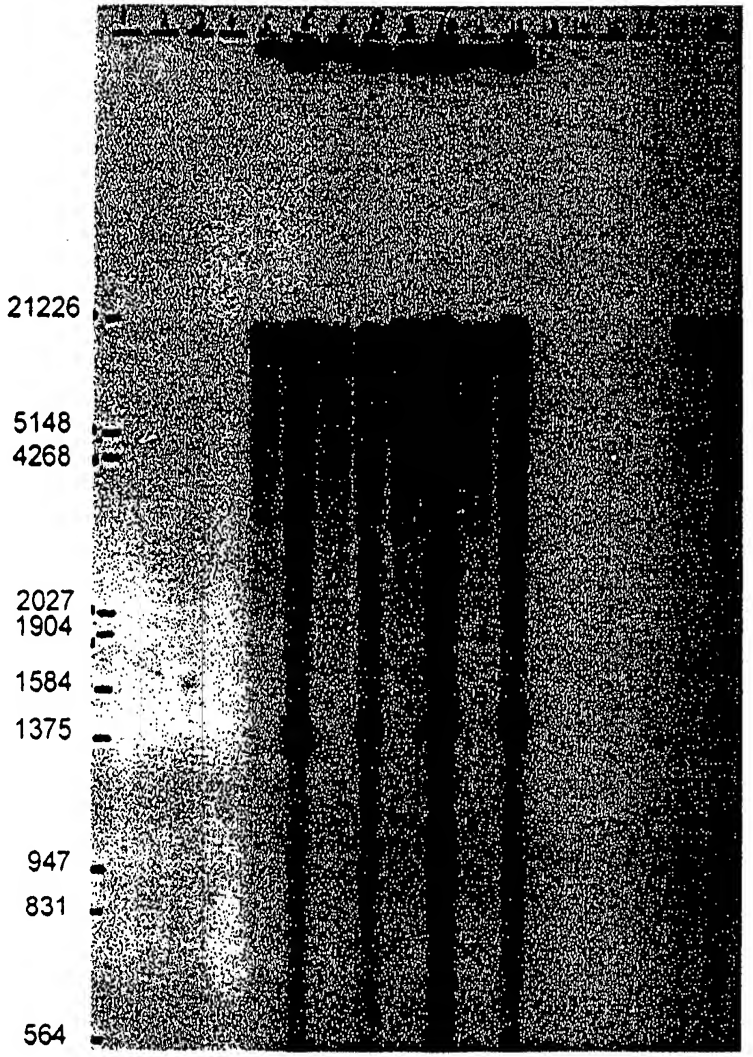


FIG. 26B

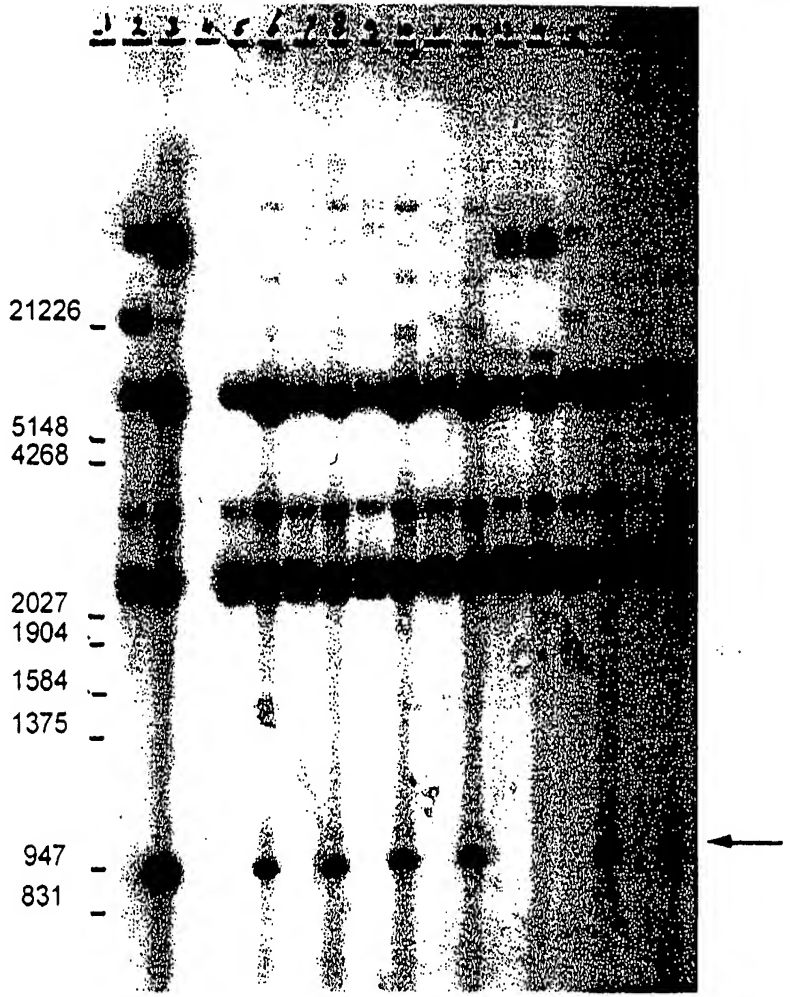
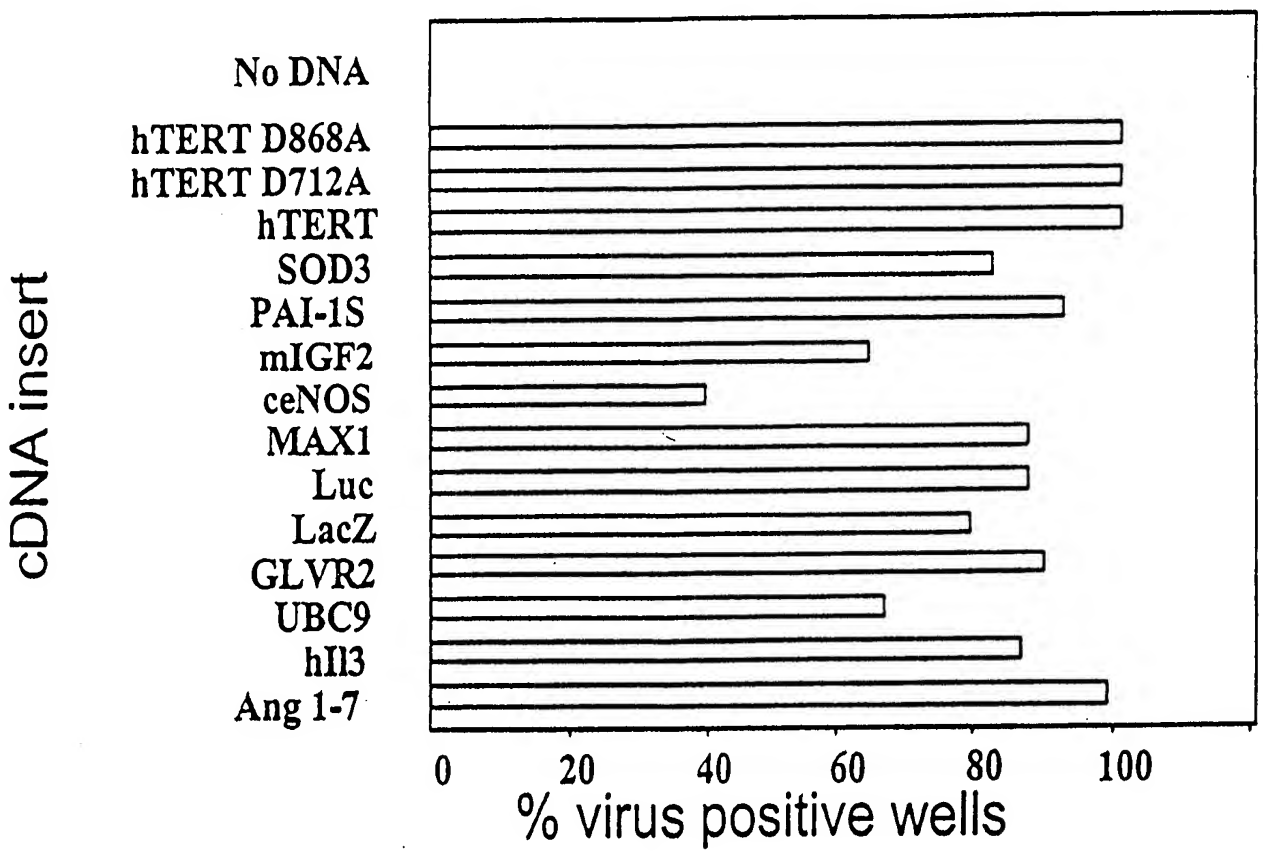


FIG. 26C



Average percentage CPE efficiency: 86 %

FIG. 27

Gene	Insert kb
• ceNOS	3.6
• hTERT	3.5
hTERT D712A	3.5
lacZ	3.2
hCAT1	2.2
• GLVR2	2.0
• Luc	1.7
• SOD3	1.4
• MAX1	.550
• hVEGF121	.511
• hIL3	.434
• UBC9	.412
ANG1-7	.104

Average titer
$0.8 \pm 0.7 \times 10^9$ pfu/ml

FIG. 28

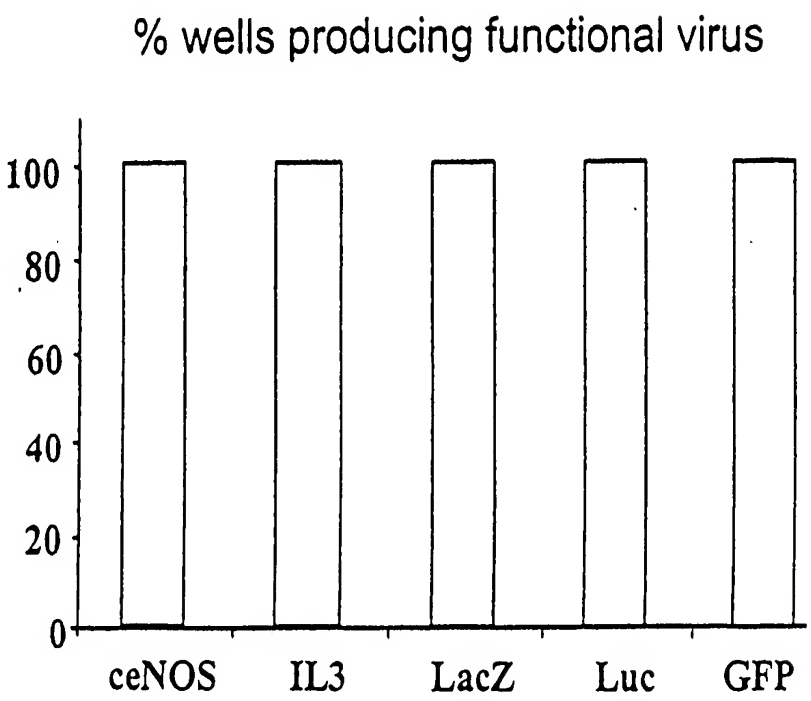


FIG. 29

Gene	Number of CPE+ wells
ceNOS	19/19
IL3	7/7
lacZ	36/36
Luc	40/40
GFP	48/48

Gene	Number of plaques
ceNOS	9/9
IL3	9/9
lacZ	40/40
Luc	9/9
EGFP	IP
GLVR2	9/9

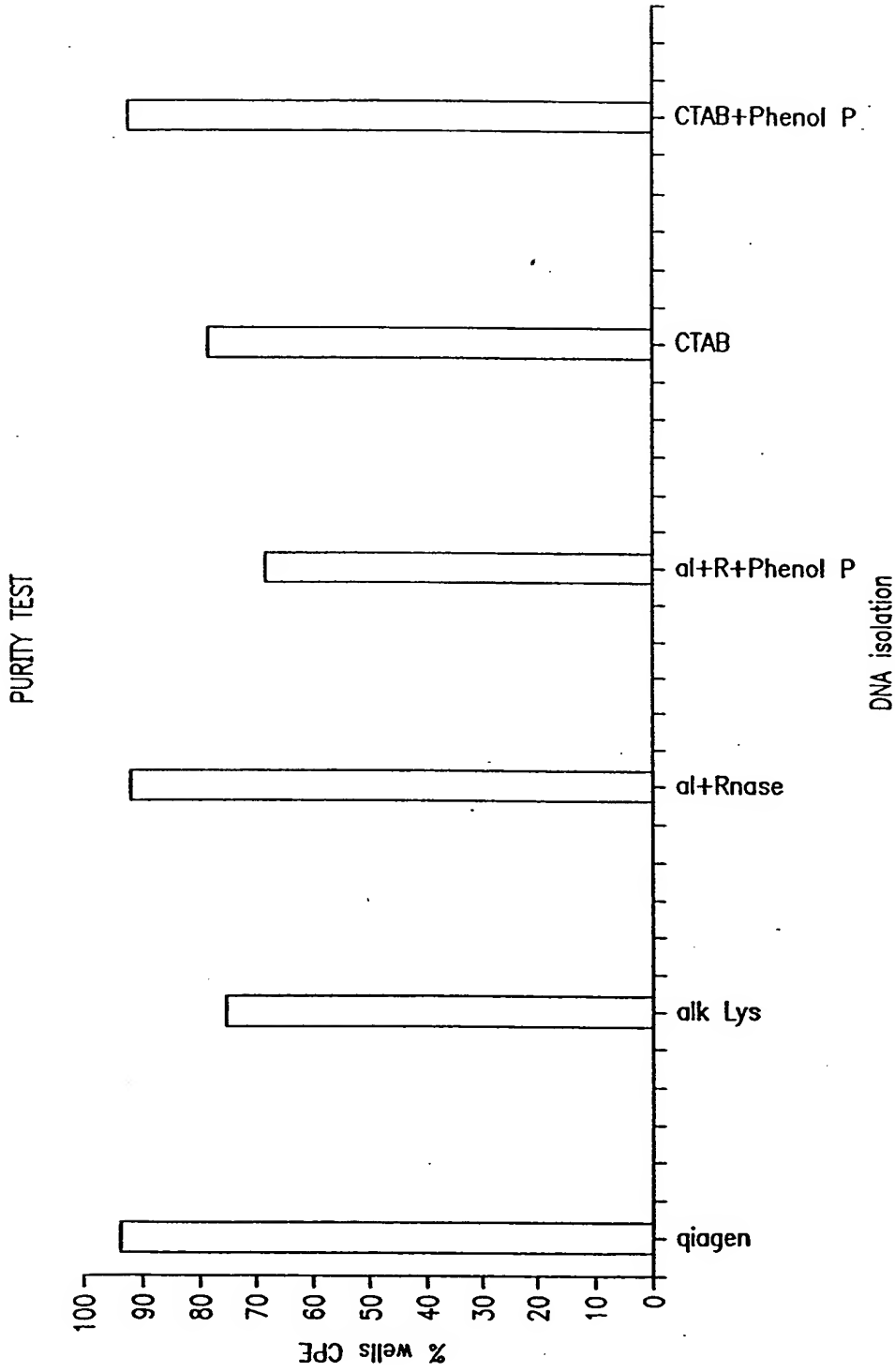


FIG. 30

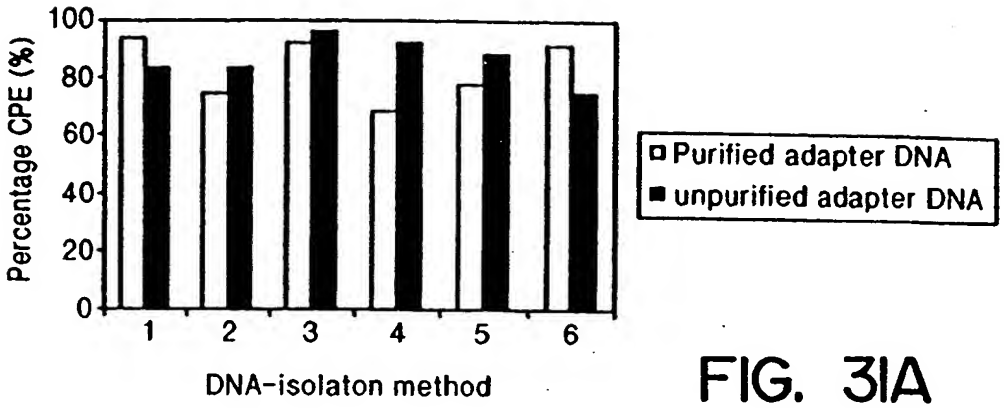


FIG. 31A

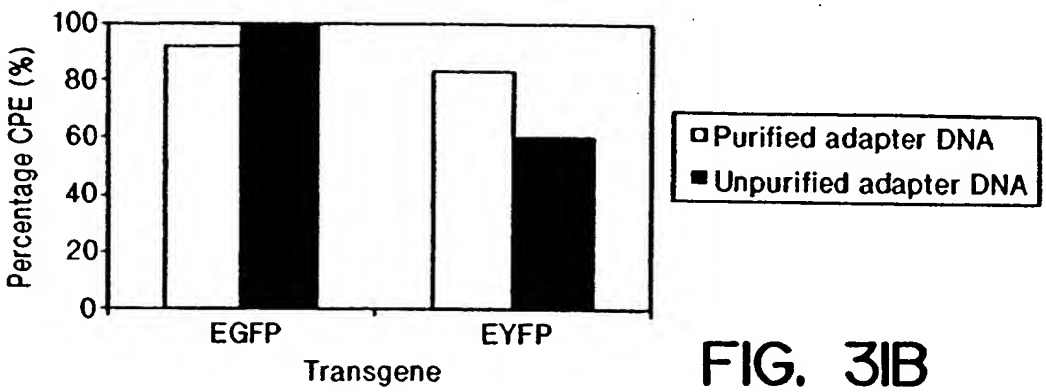


FIG. 31B

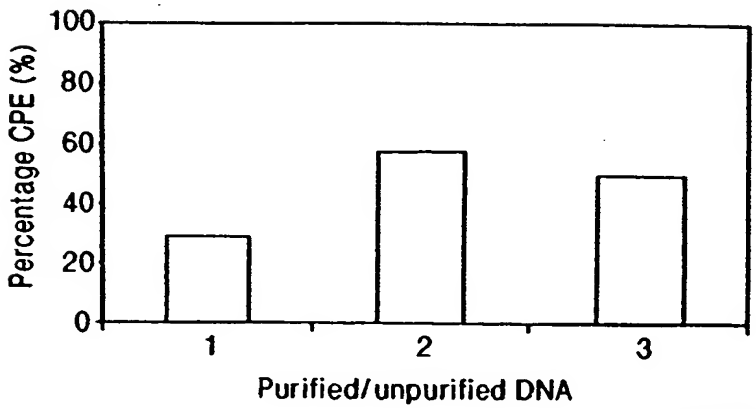
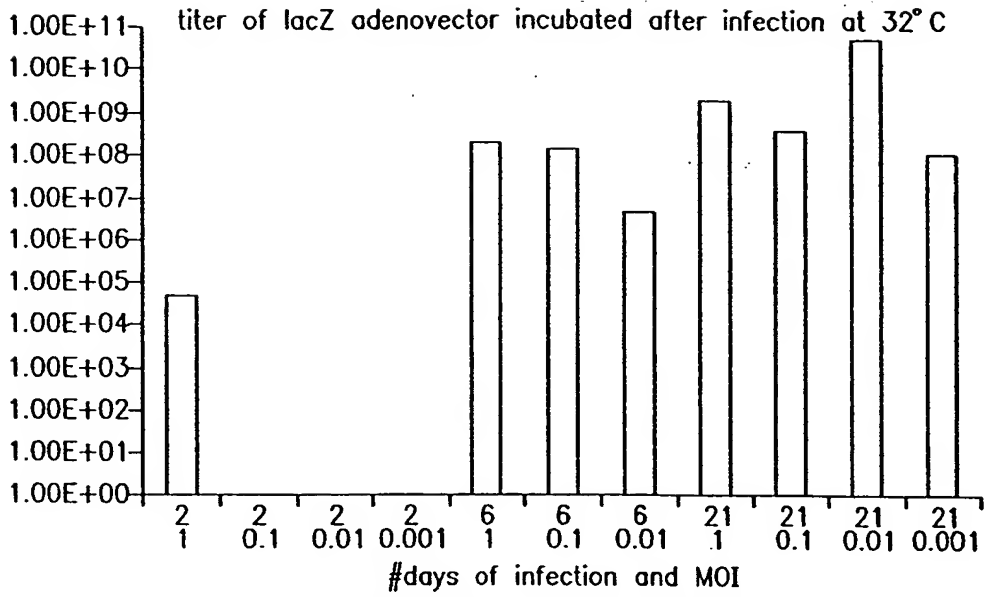
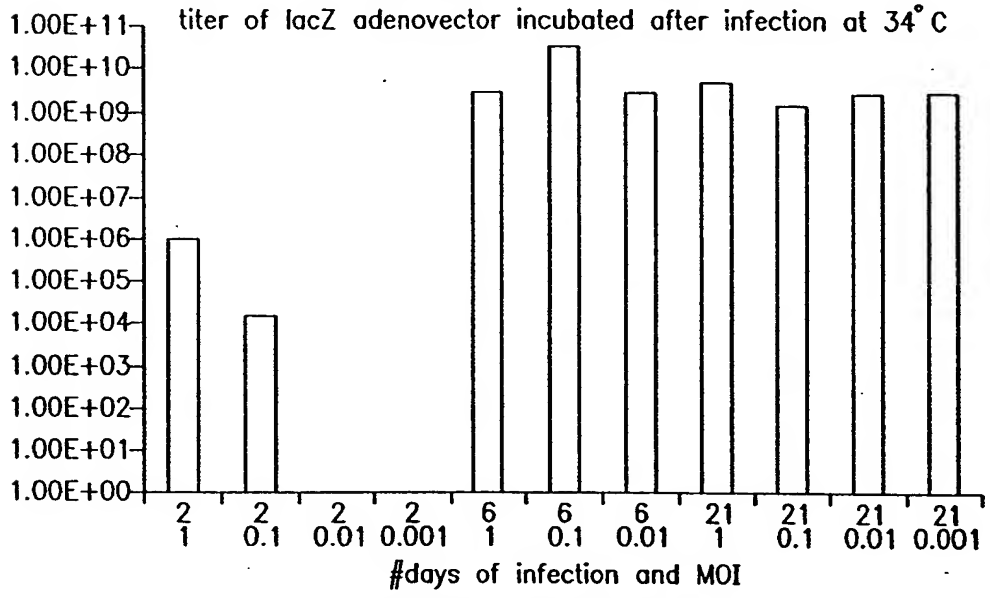


FIG. 31C





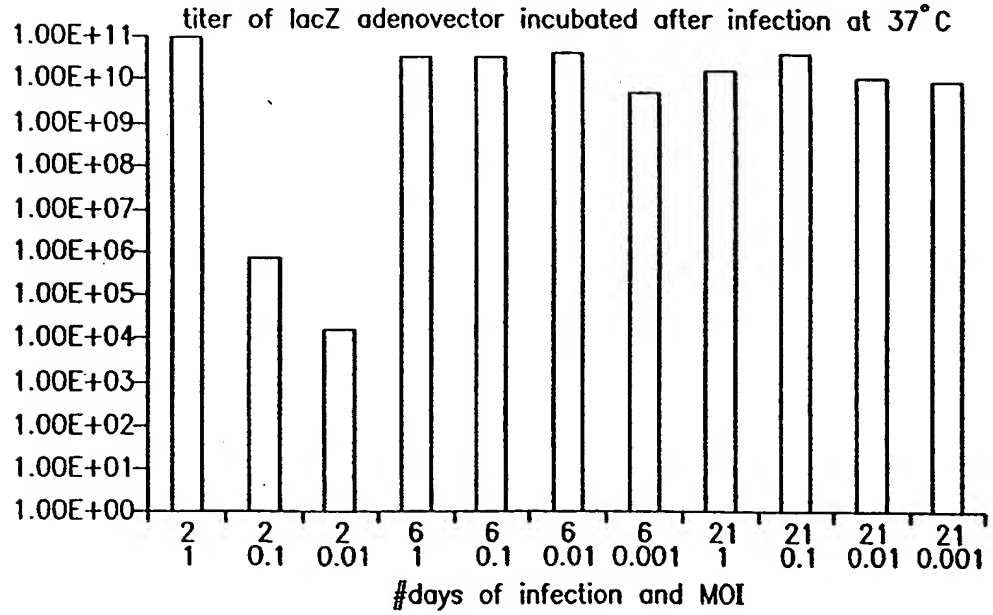


FIG. 32

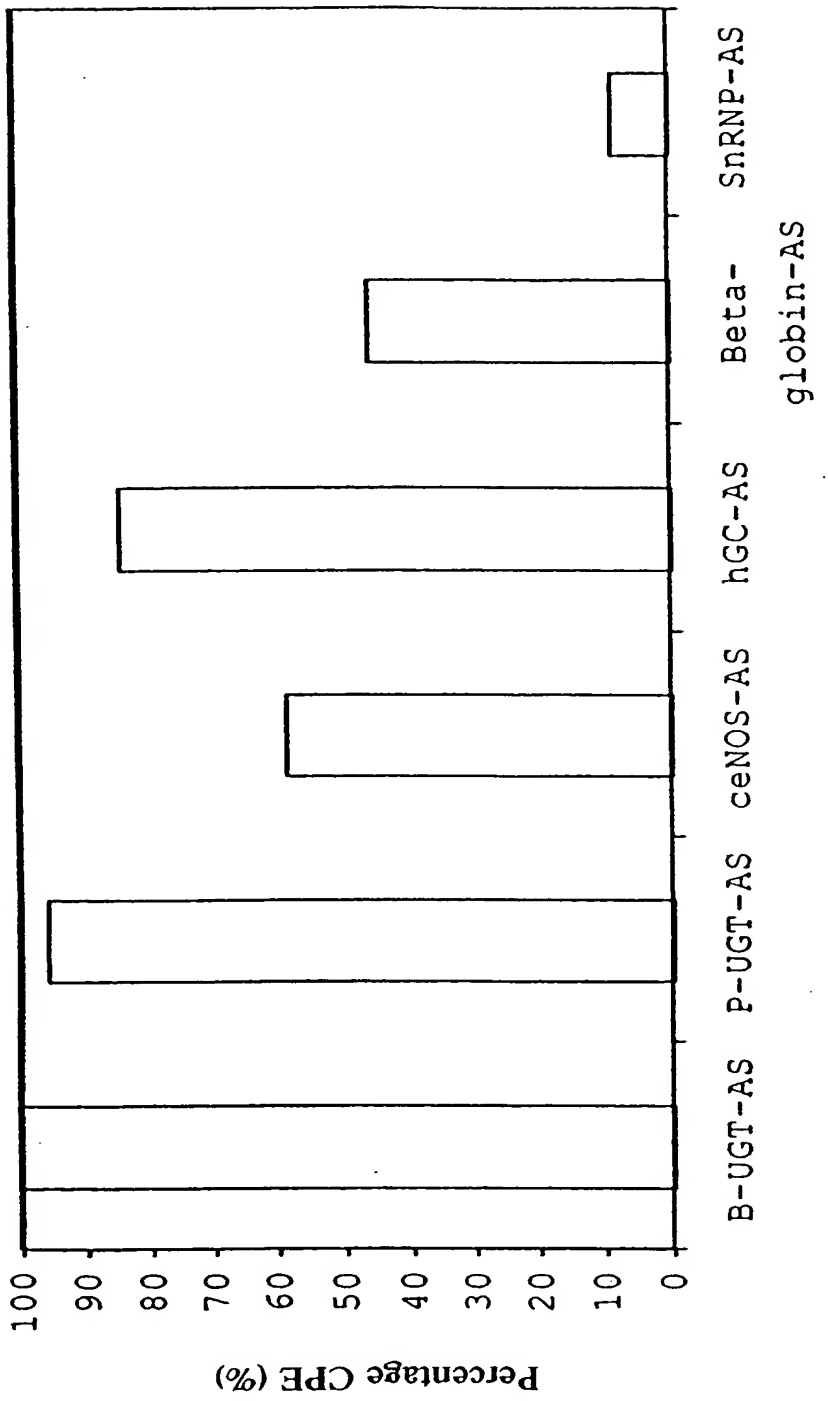


FIG. 33

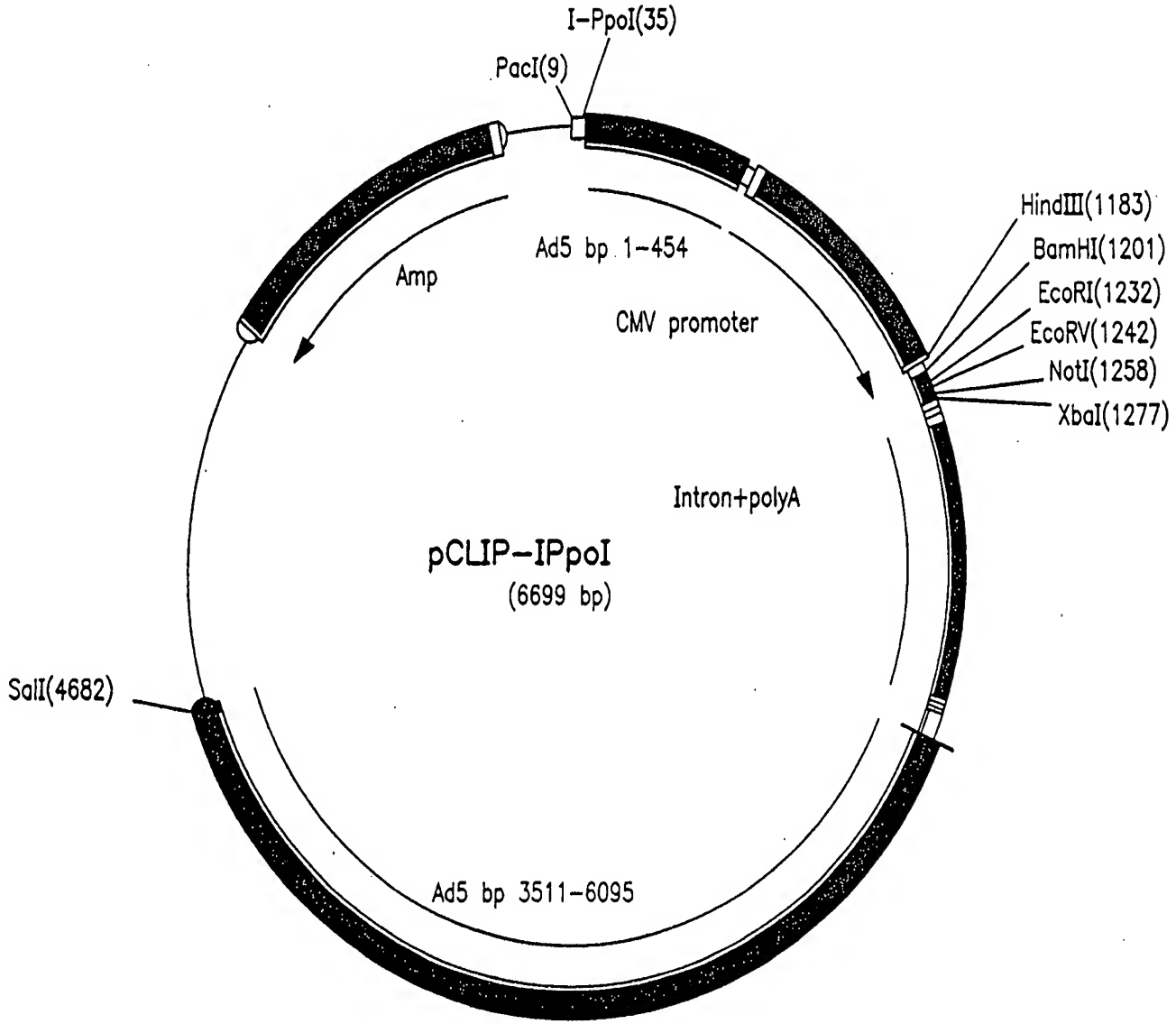


FIG. 34A

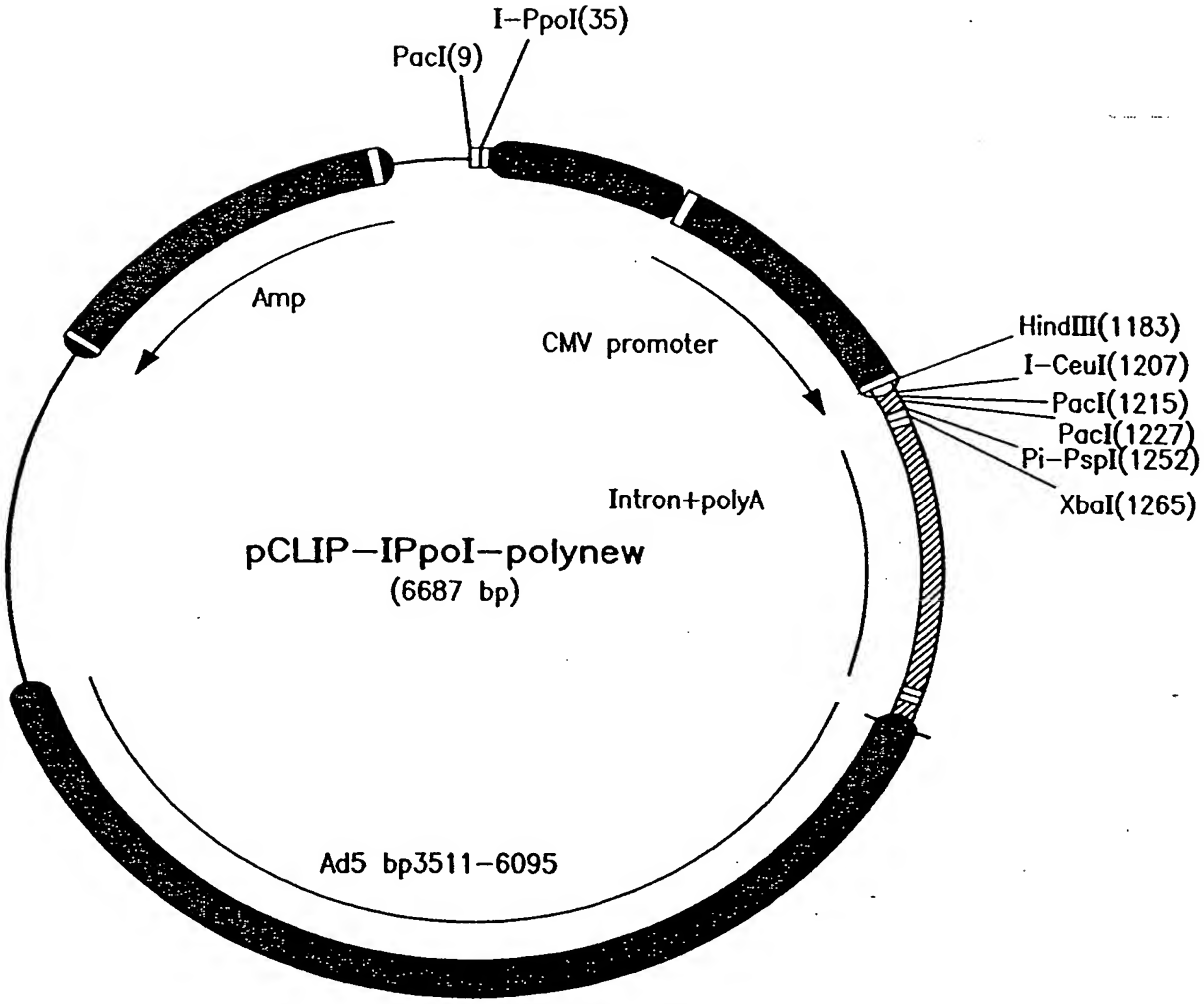


FIG. 34B

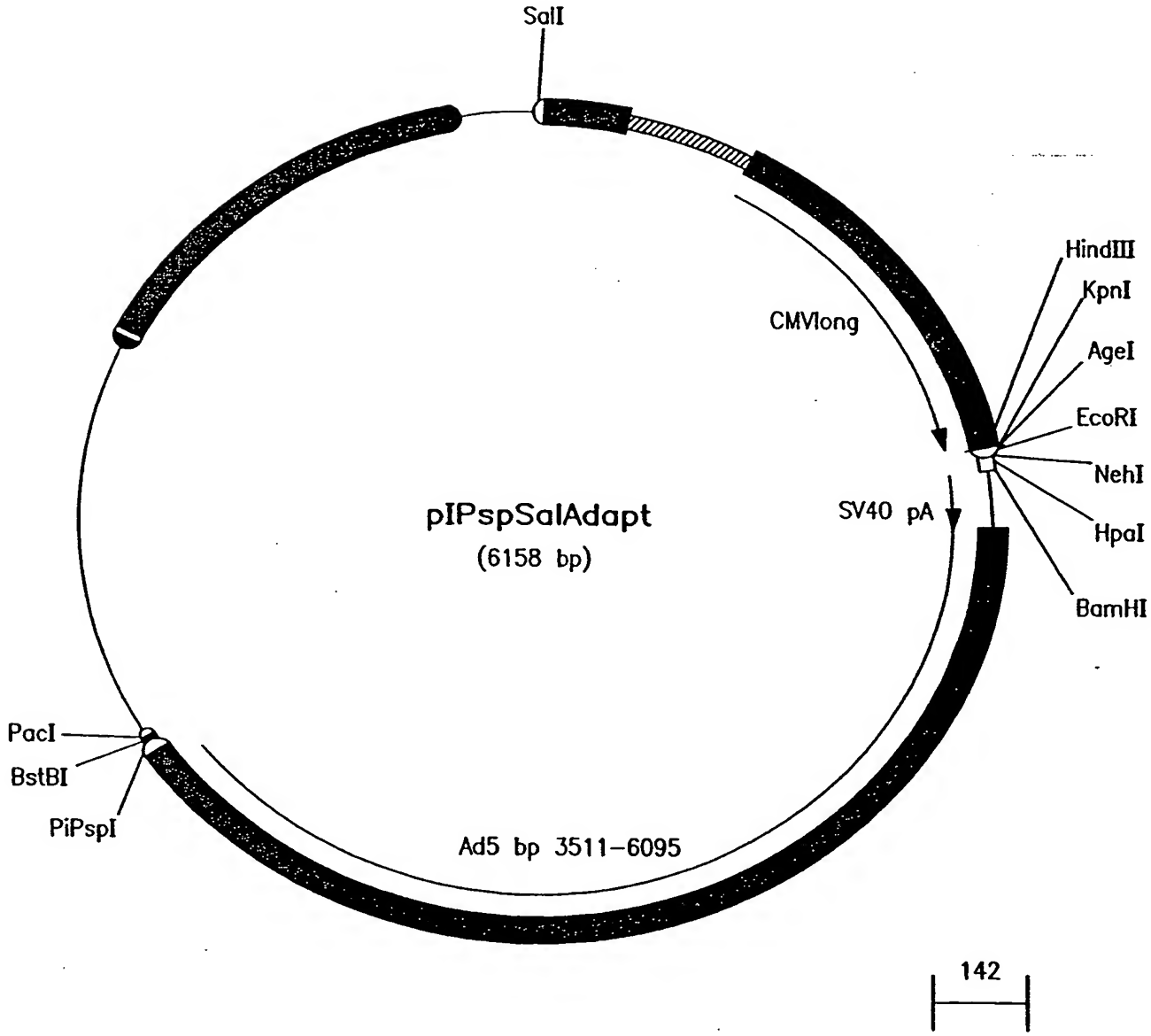


FIG. 34C

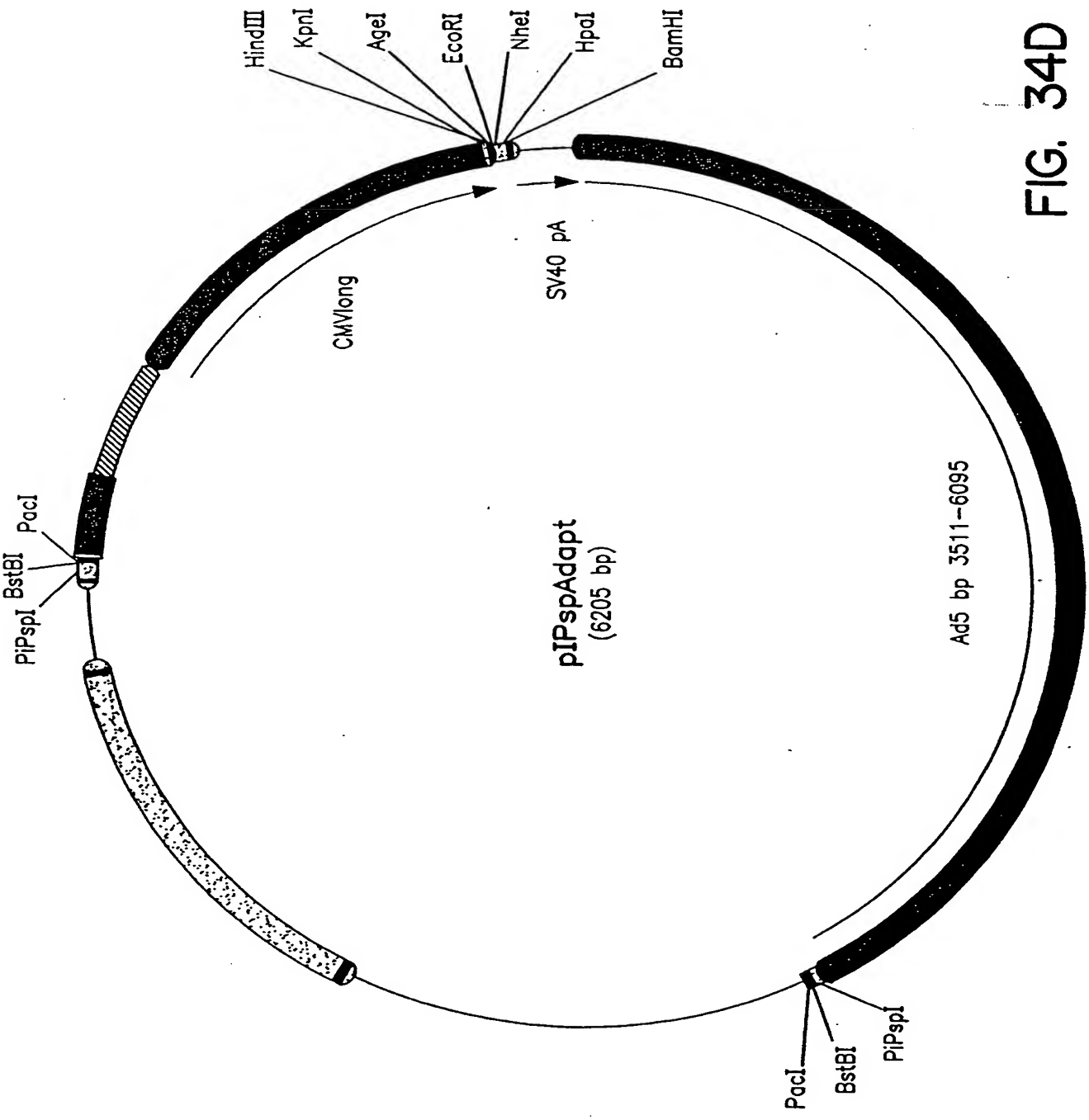


FIG. 34D

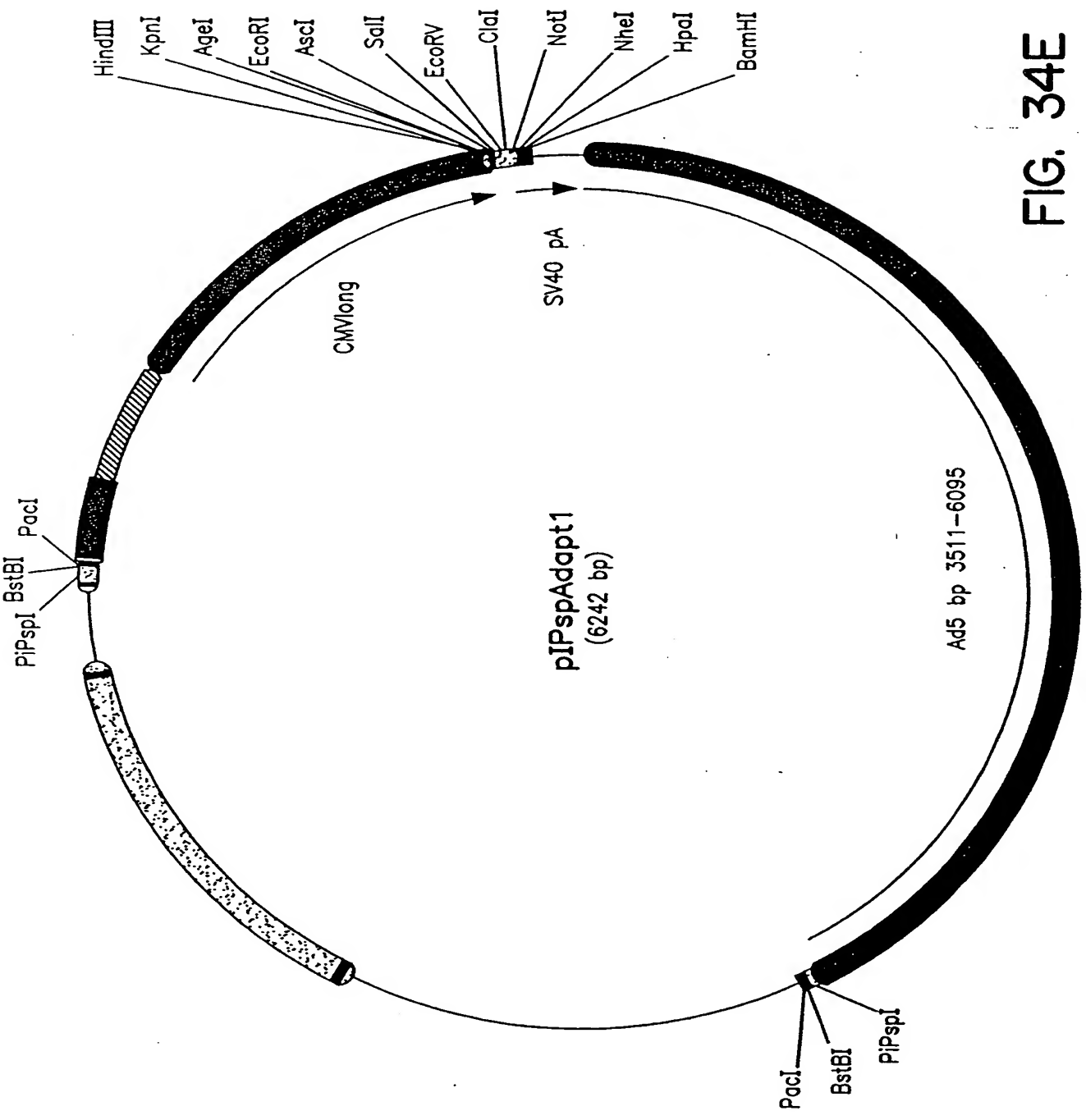


FIG. 34E

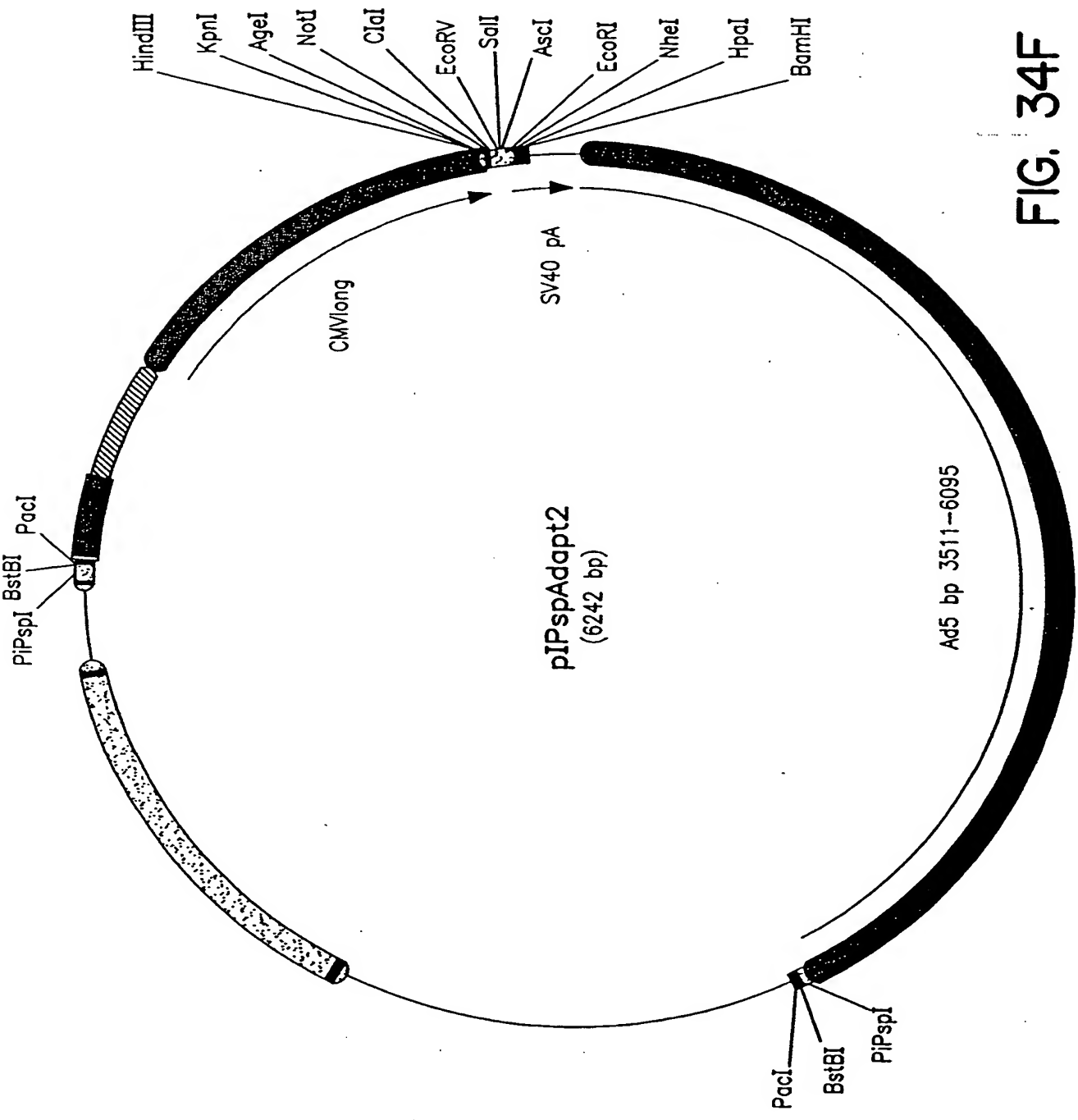


FIG. 34F

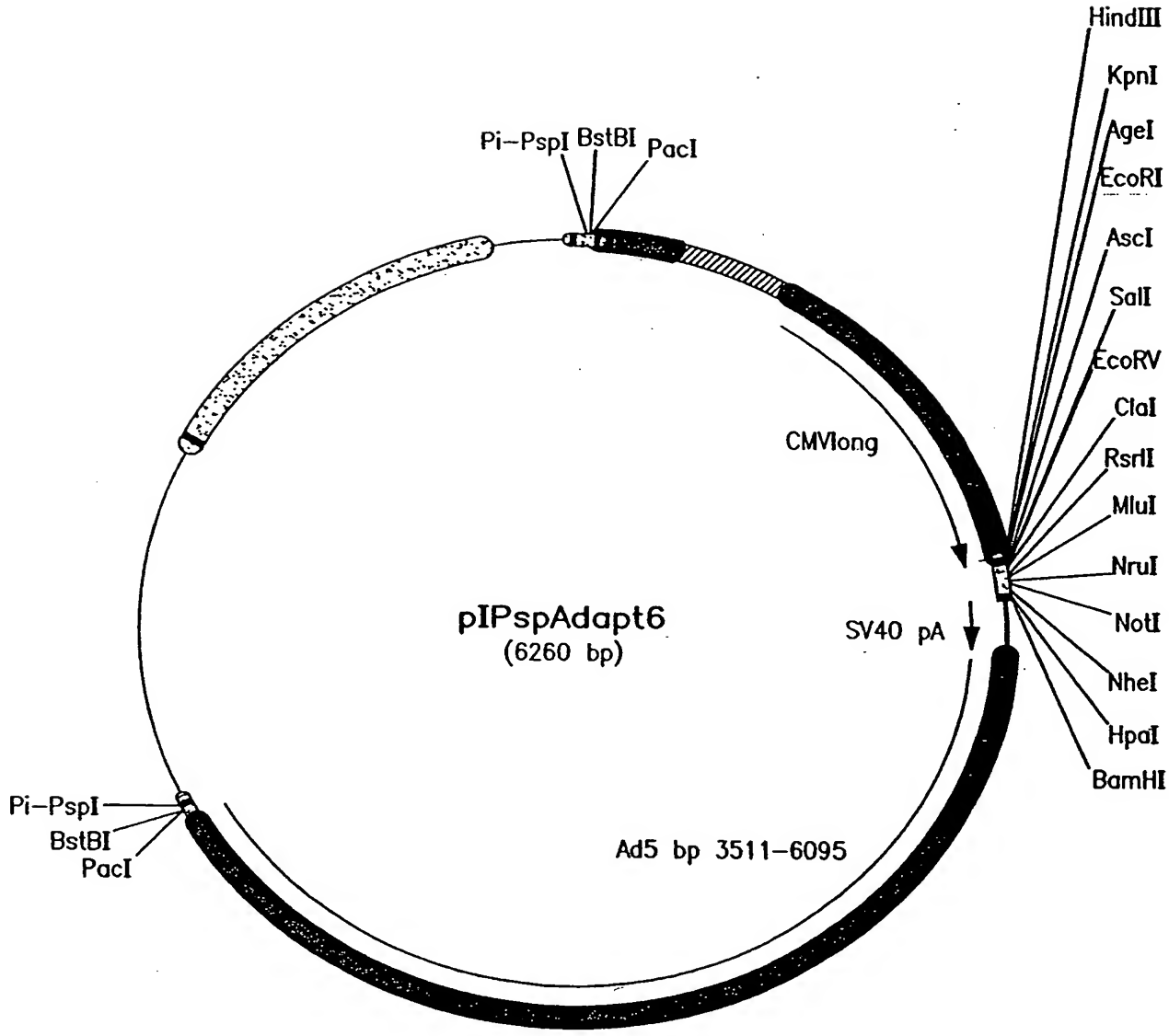


FIG. 34G

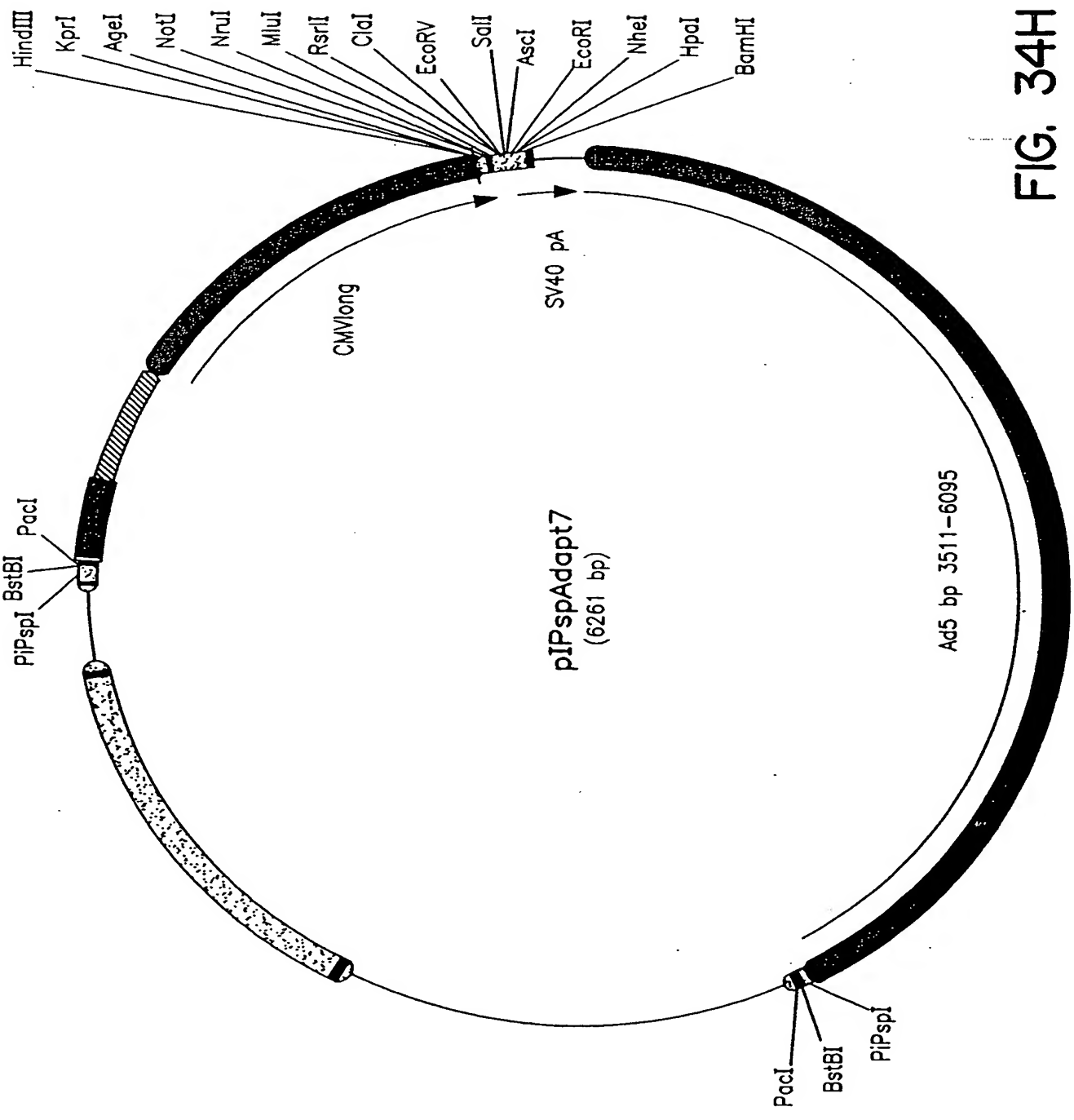


FIG. 34H

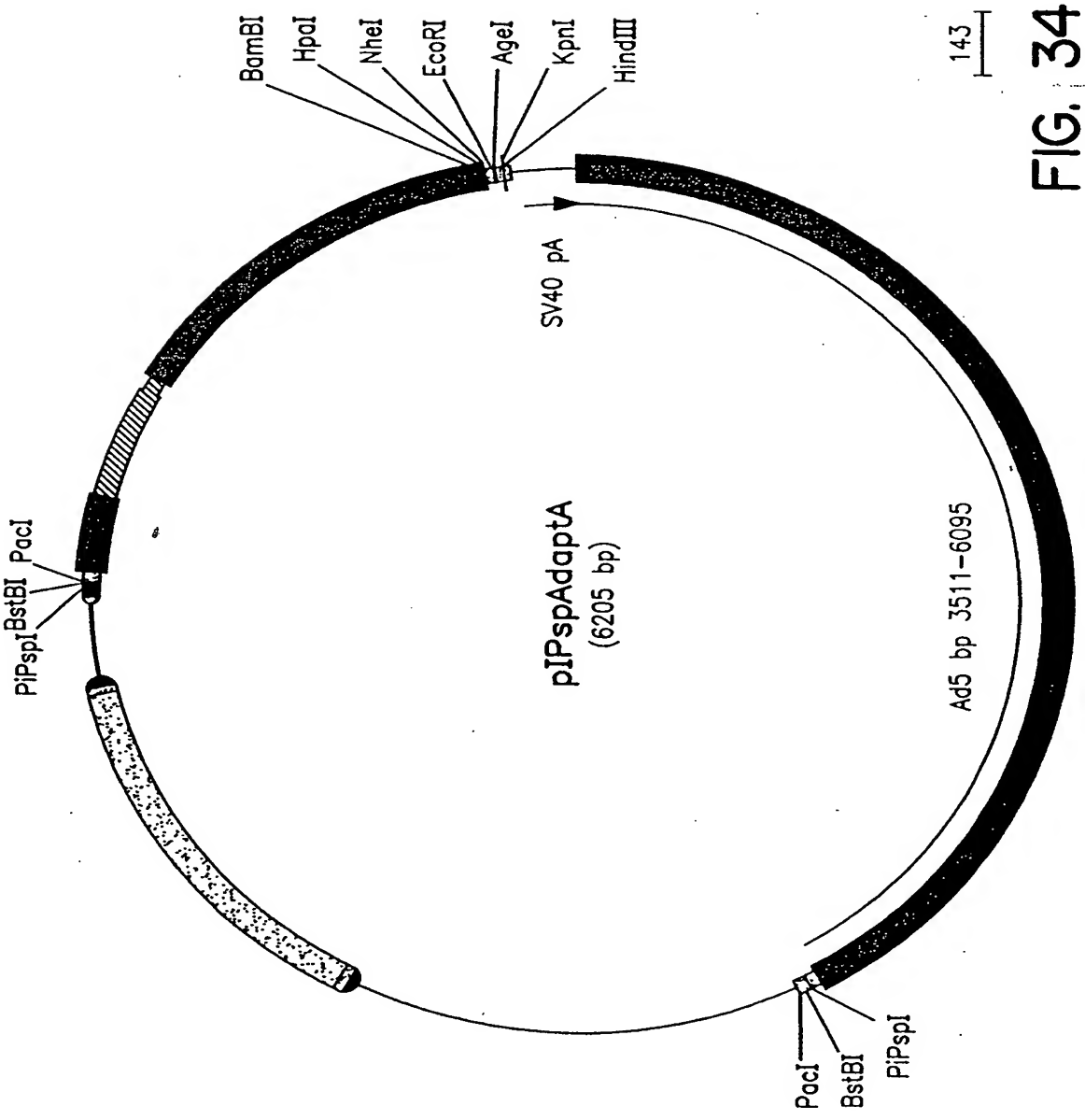


FIG. 34 I

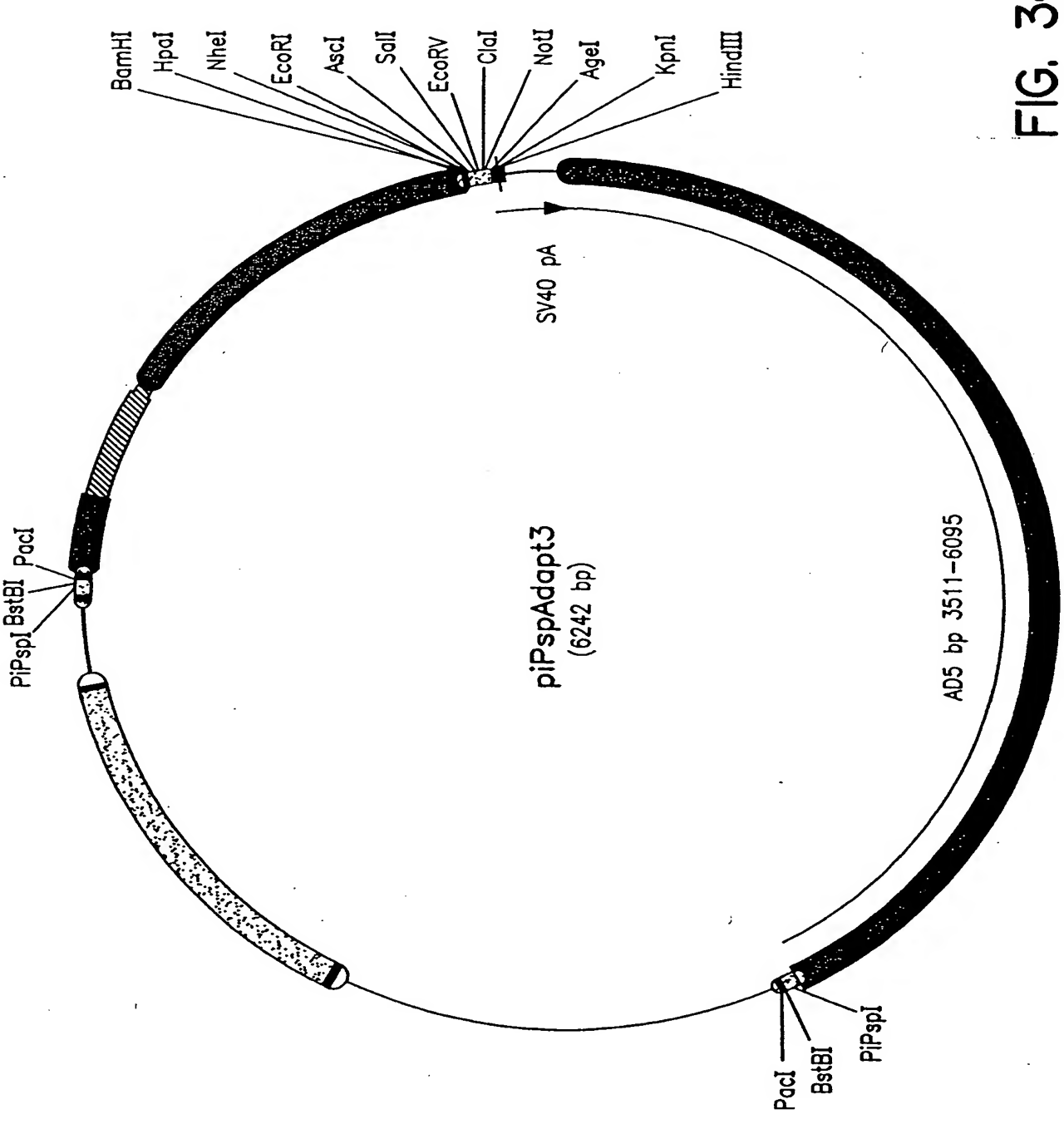


FIG. 34J

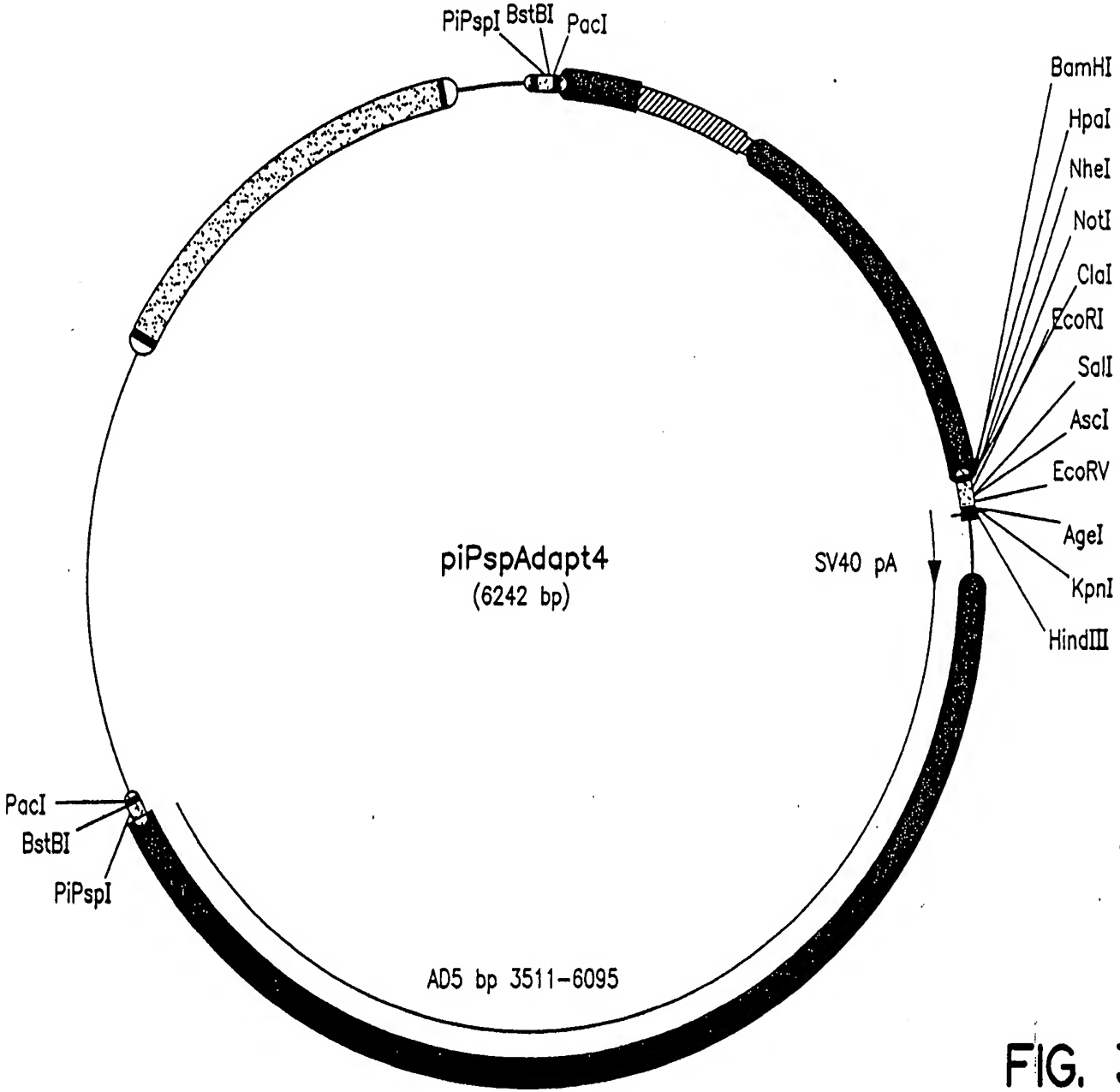


FIG. 34K

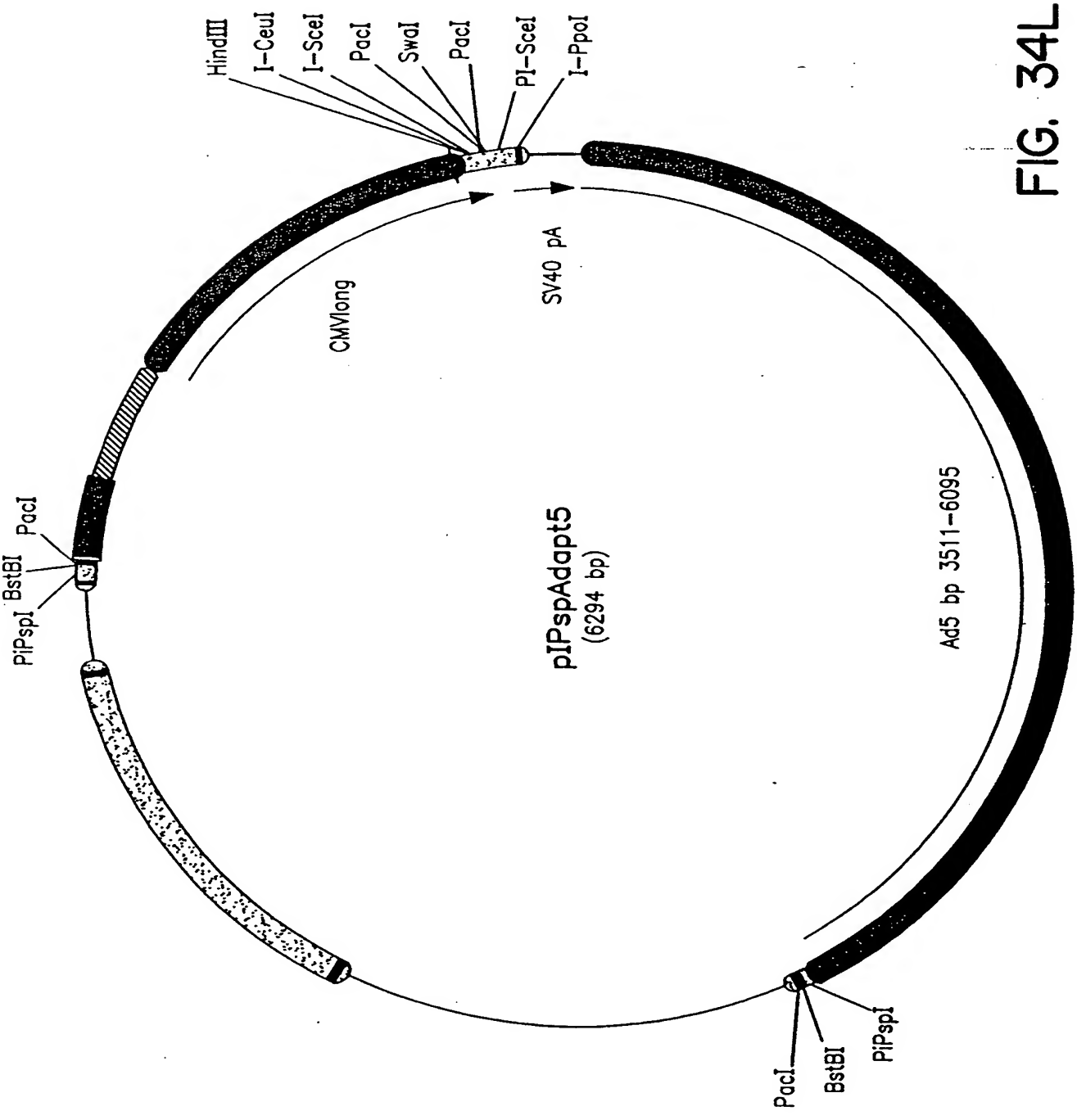


FIG. 34L

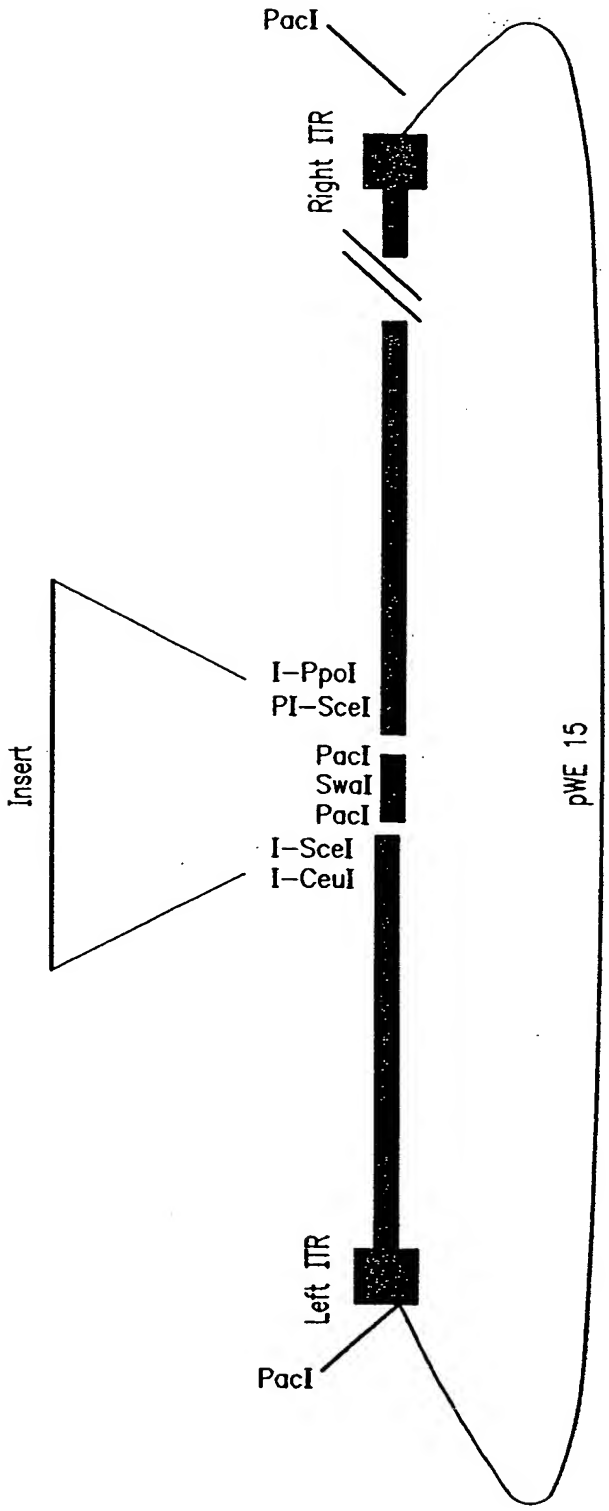


FIG. 34M

Relative amounts of wells with CPE after transfection of PER.C6/E2A cells with pCLIP–LacZ and the adapter plasmid pIPspAdapt2.

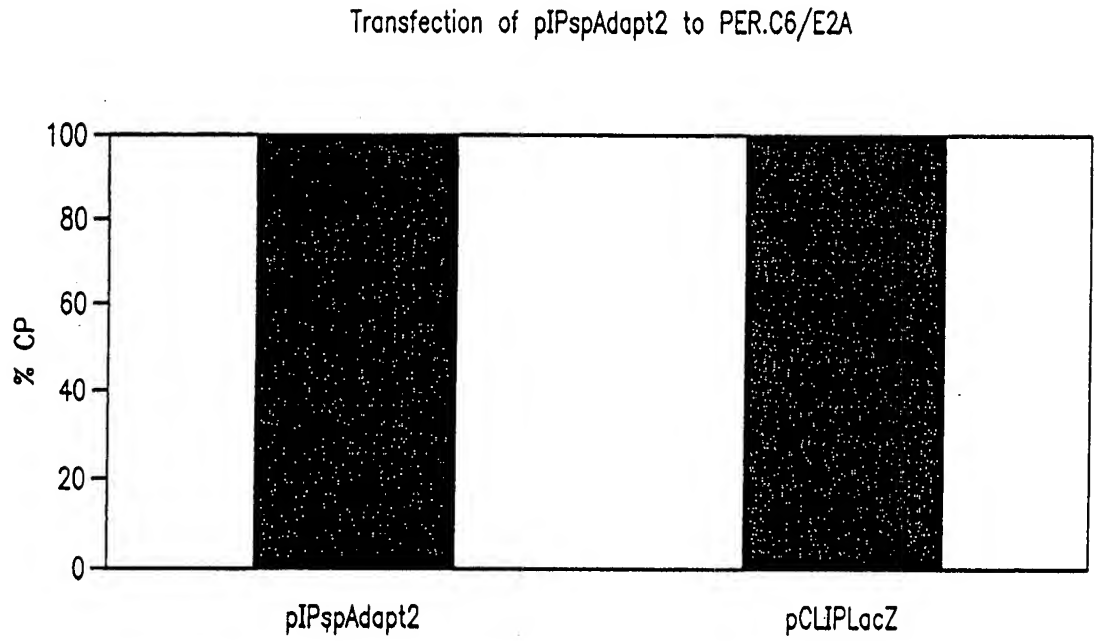


FIG. 34N

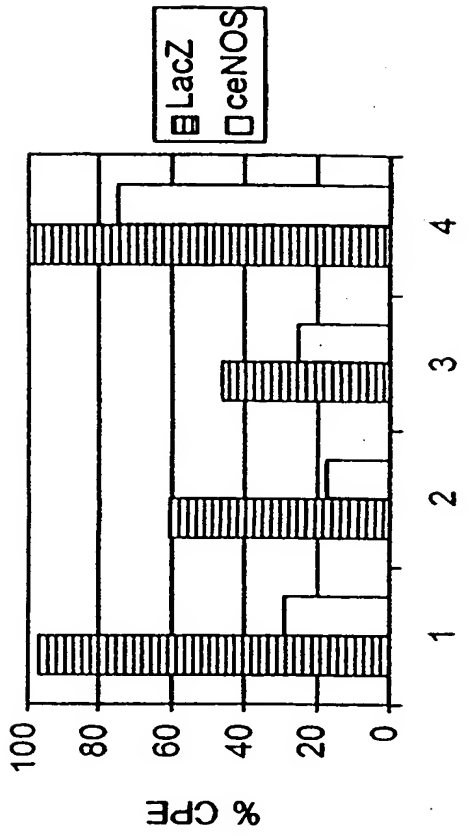


FIG. 35

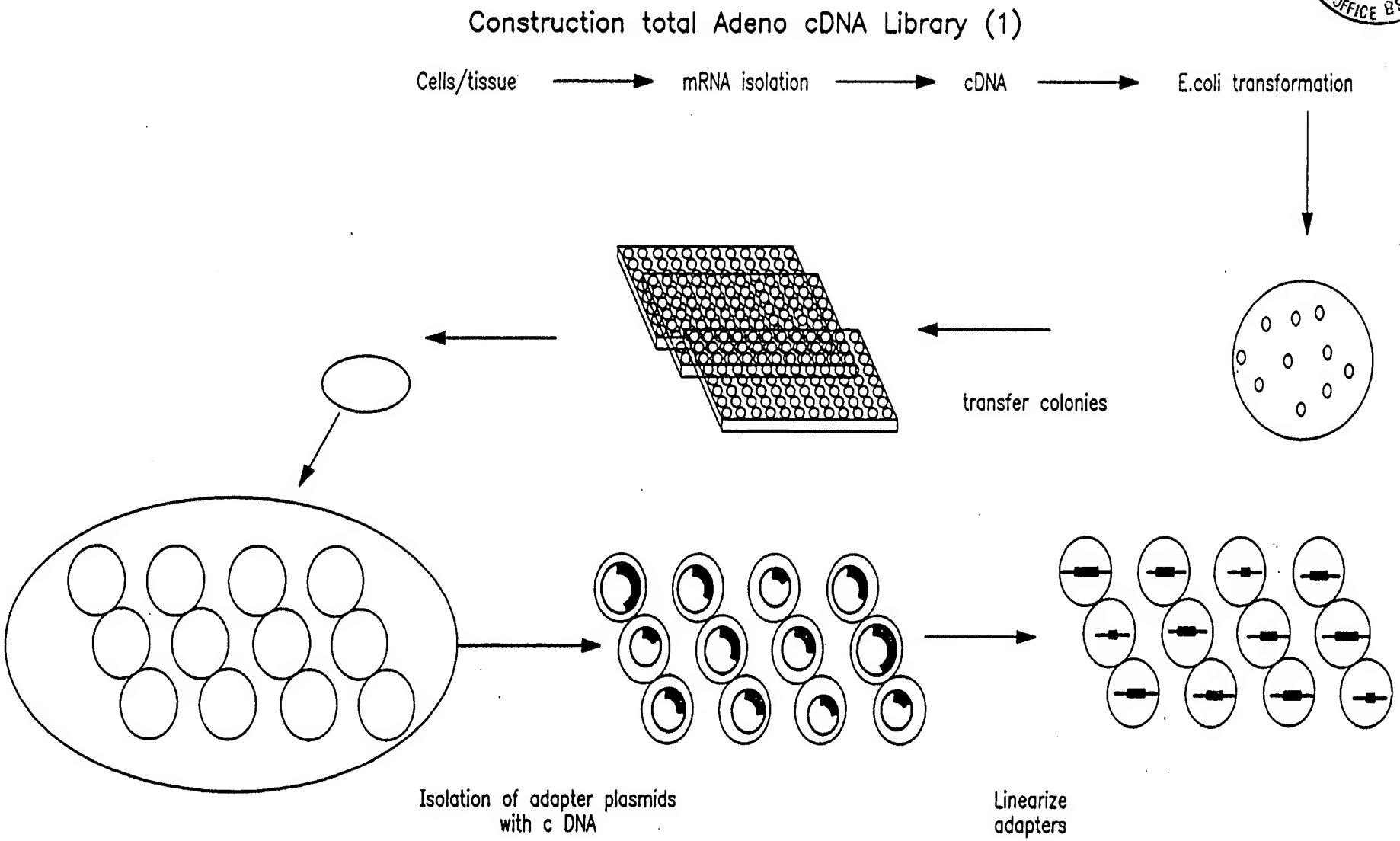


FIG. 36A

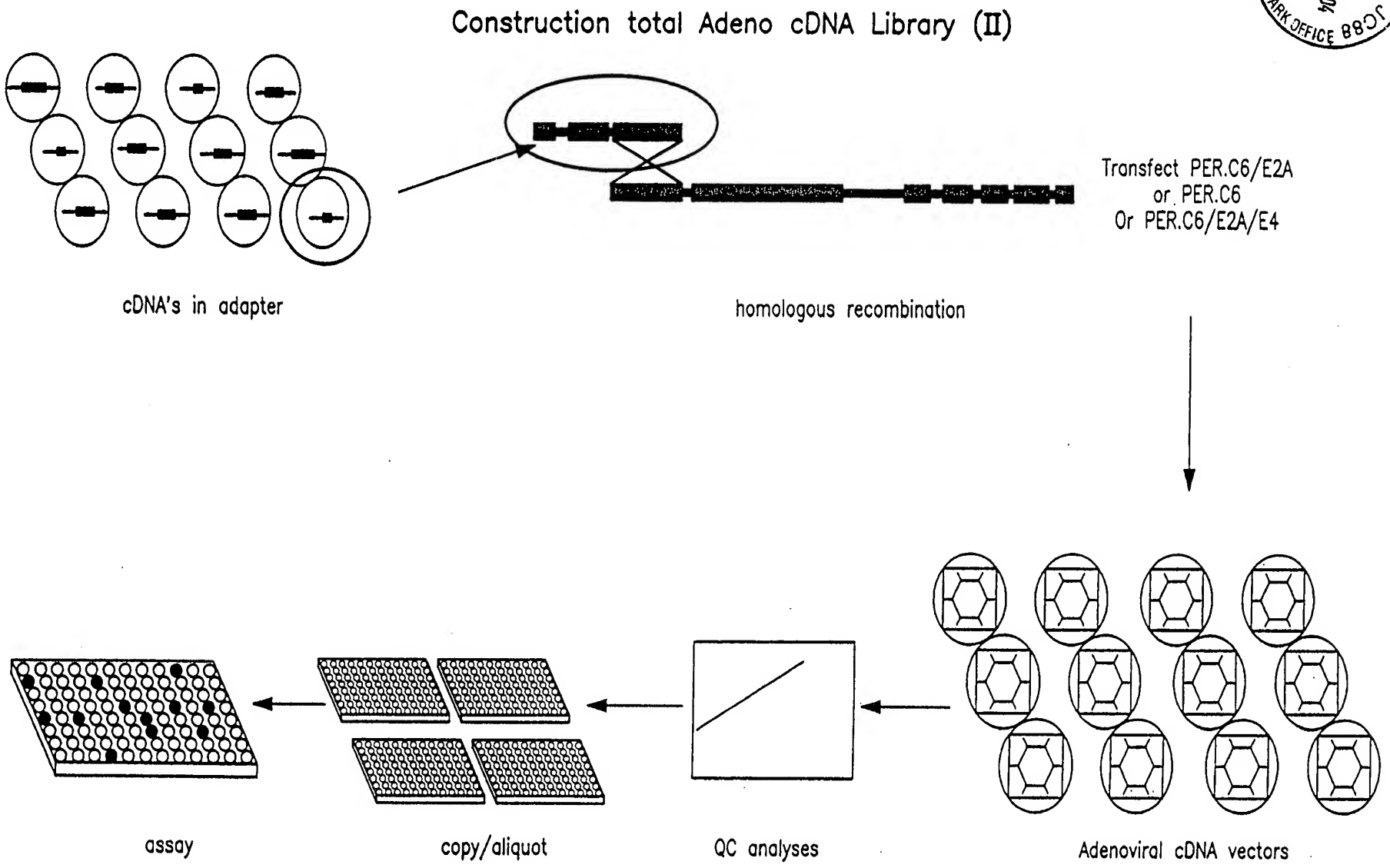


FIG. 36B

EXAMPLE 21 384 WELL PLATE IN PROGRESS

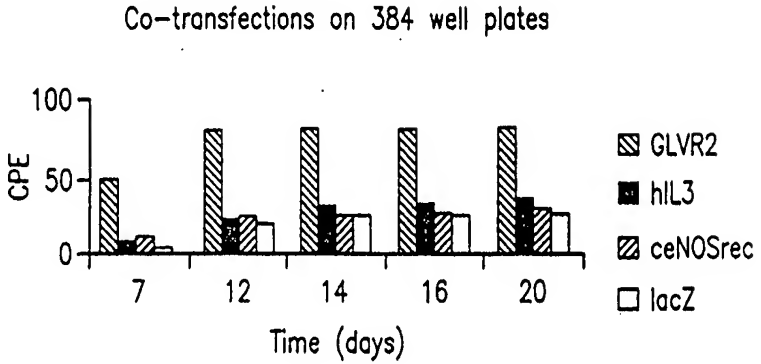


FIG. 37A

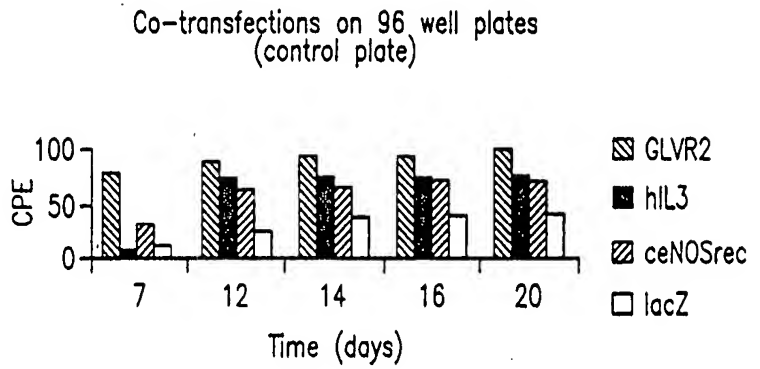


FIG. 37B

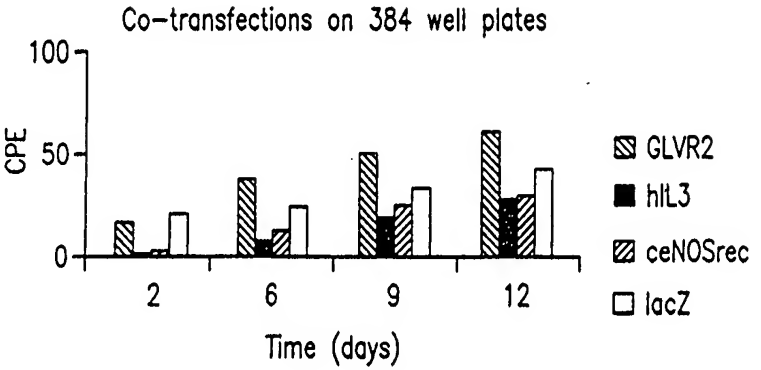


FIG. 37C

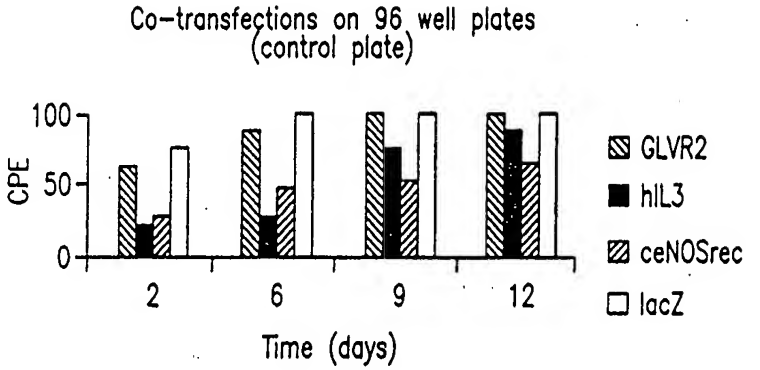


FIG. 37D

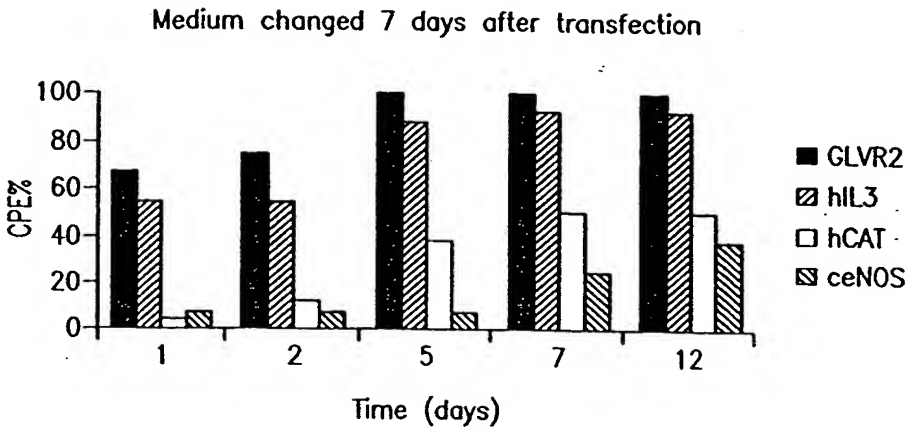


FIG. 38A

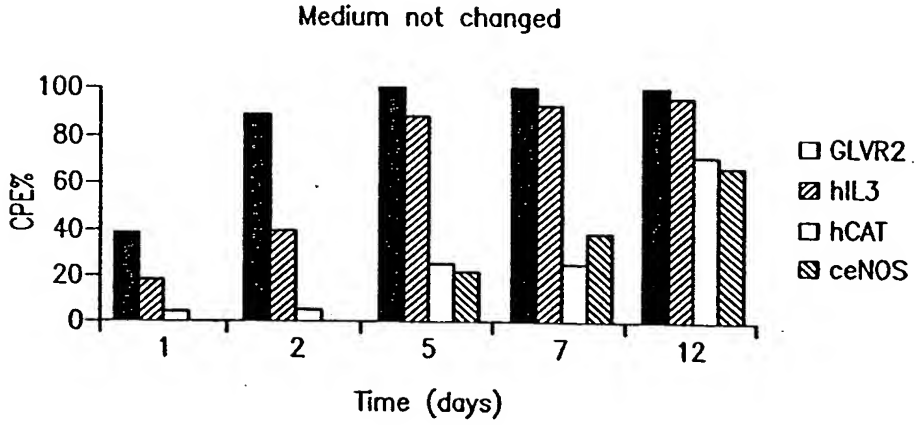


FIG. 38B

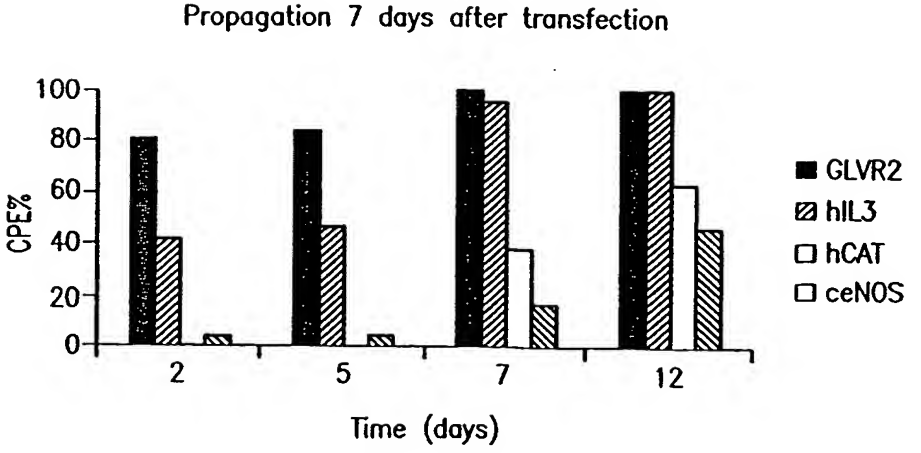


FIG. 38C

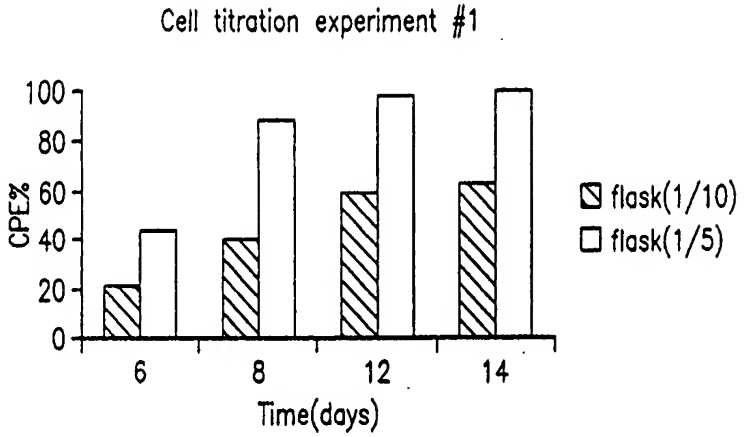


FIG. 39A

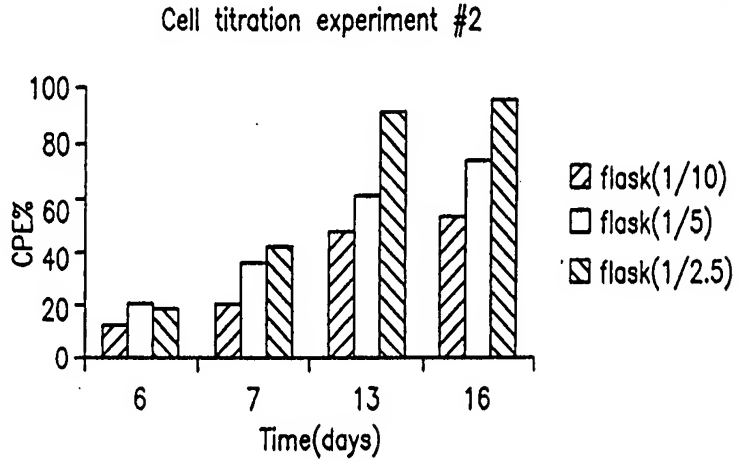


FIG. 39B

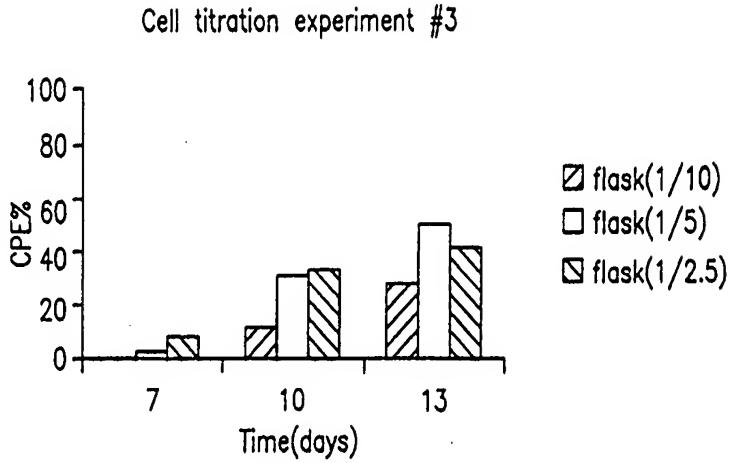


FIG. 39C

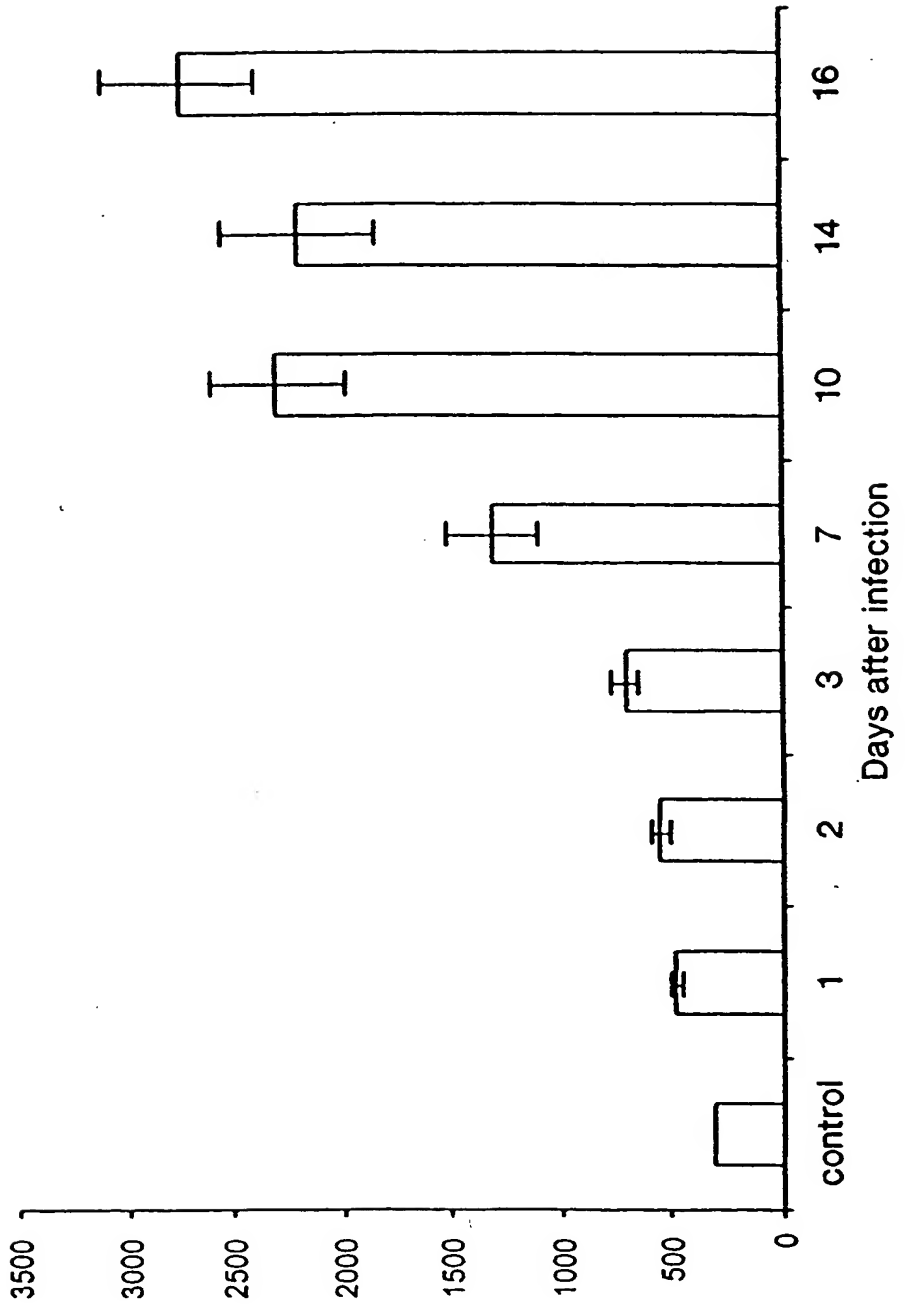


FIG. 40

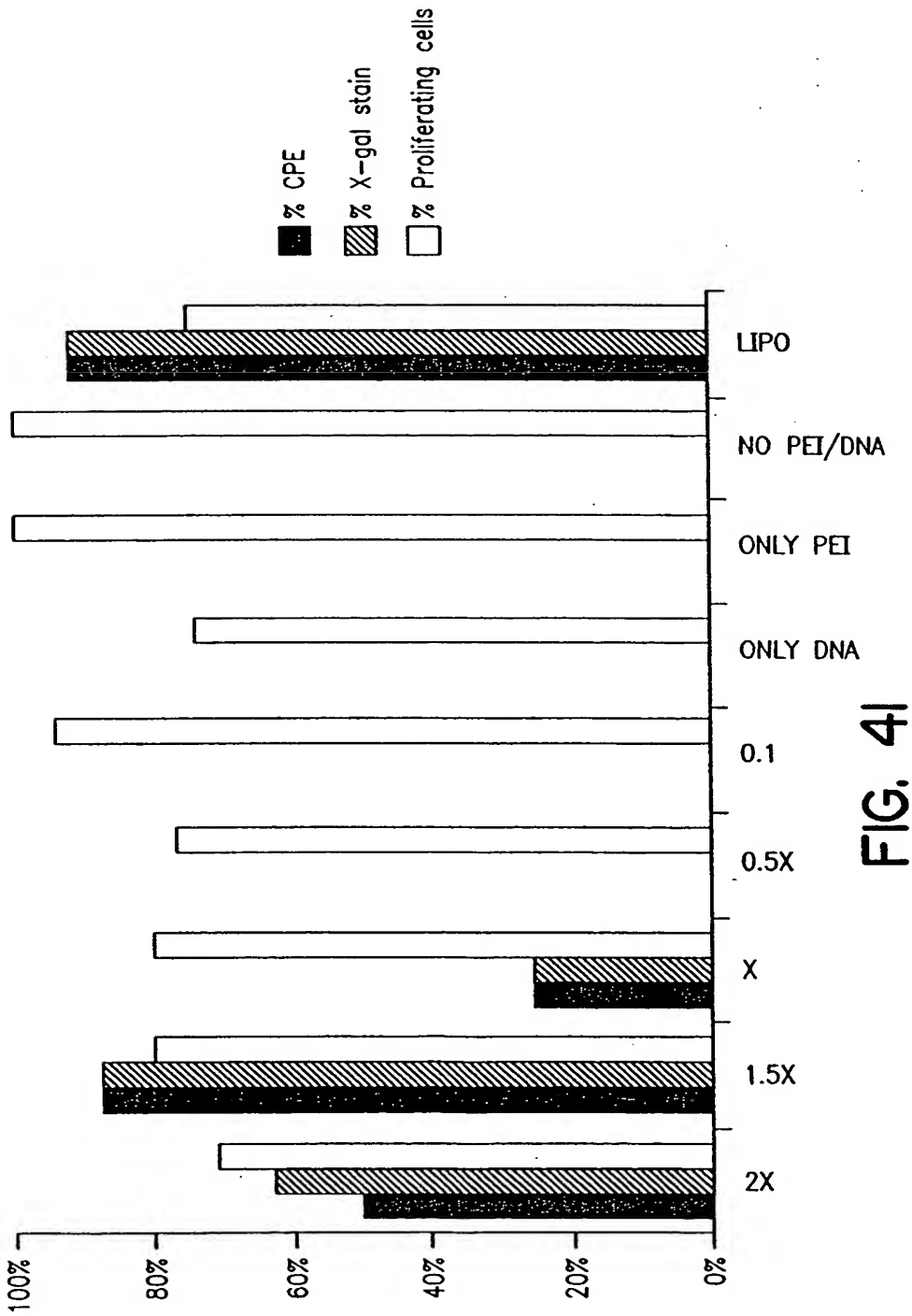


FIG. 4I

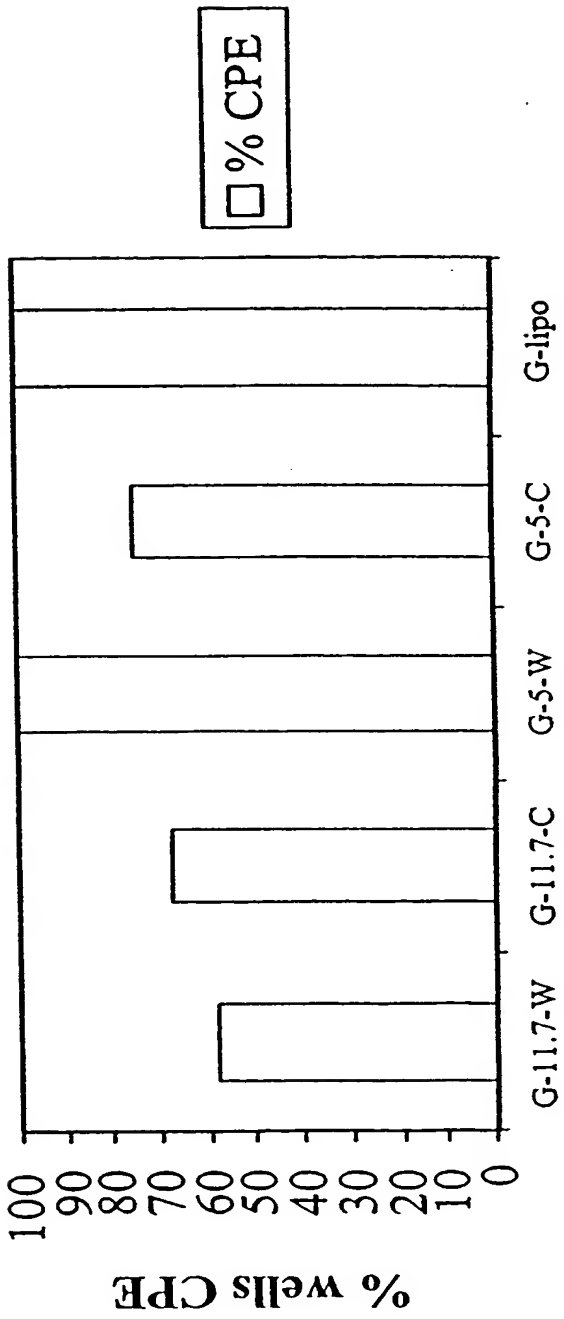


FIG. 42

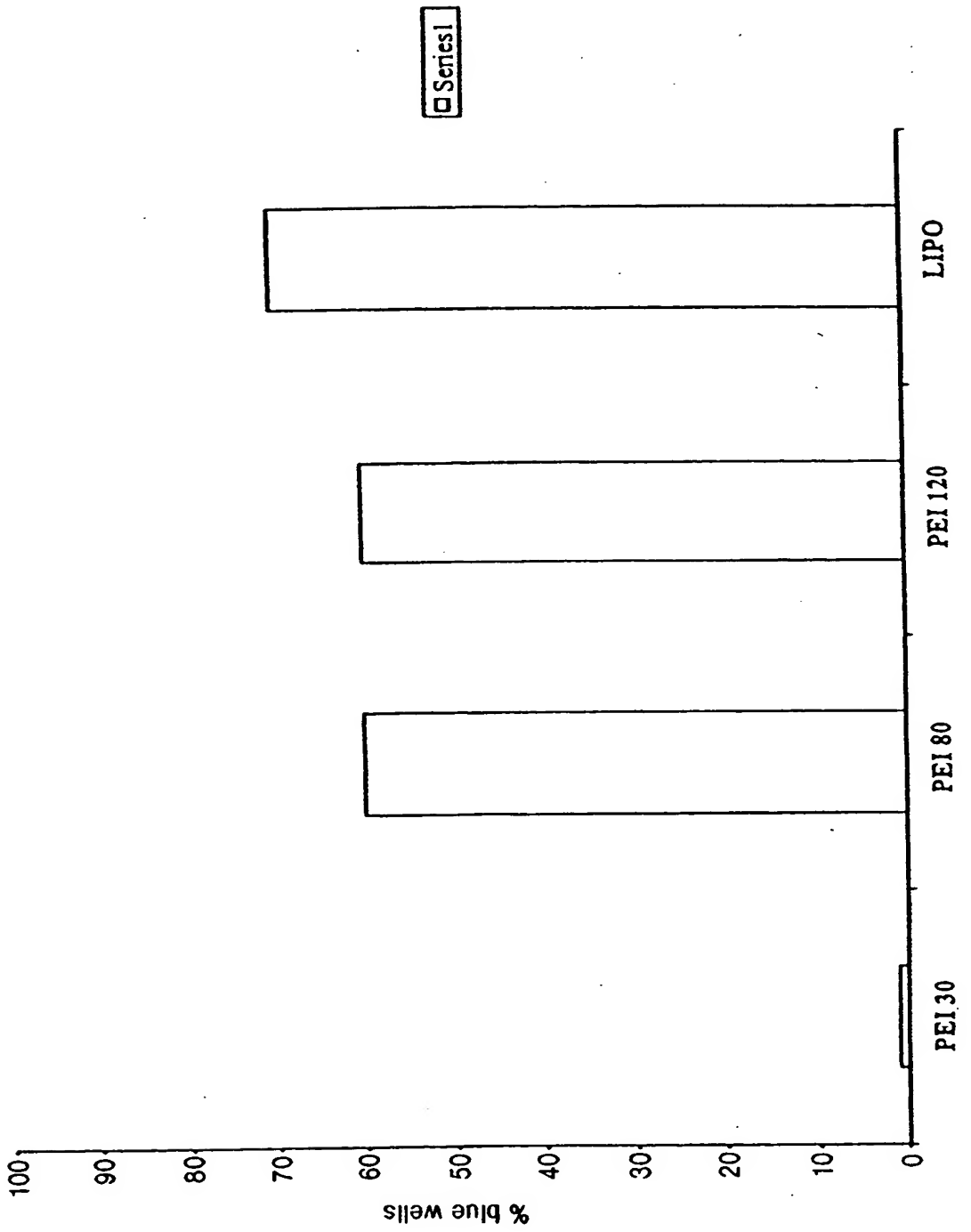


FIG. 43

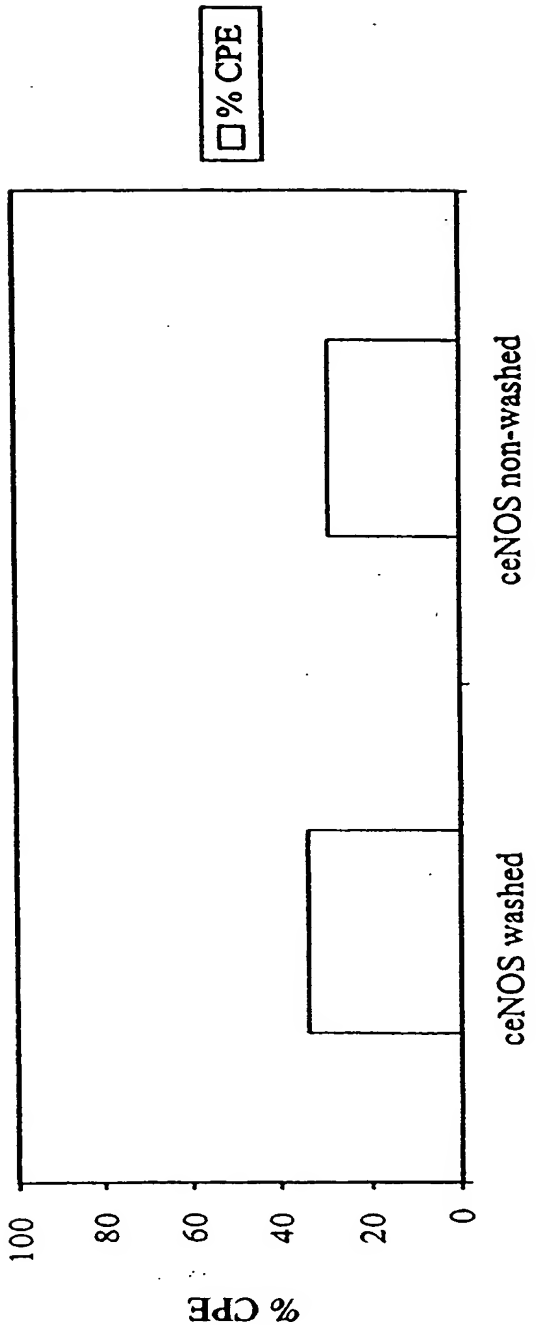


FIG. 44